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Foreword

The National Toxicology Program (NTP) was established in 1978 by the Secretary of Health and Human Services to coordinate toxicology research and testing of potentially hazardous chemicals. The NTP is composed of sections of several Federal agencies that perform toxicology research. Members include the National Institute of Health's National Institute of Environmental Health Sciences, the Center for Disease Control's National Institute for Occupational Safety and Health, and the Food and Drug Administration's National Center for Toxicological Research. The NTP supports national public health programs by initiating research designed to understand the physiological, metabolic and genetic basis of chemical toxicity. Among the responsibilities of NTP are in vivo and in vitro toxicity testing of suspected chemicals; broadening the spectrum of toxicological information on known hazardous chemicals; developing and validating toxicologic assay systems and rapidly communicating test results to government agencies with regulatory responsibilities as well as to medical and scientific communities.

The concept for the systematic investigation of chemicals for carcinogenesis in animals as a surrogate for human risk was formulated in 1960 by the Director of the National Cancer Institute, Dr. Kenneth Endicott. The major goal was to "gather facts and acquire knowledge leading to the clarification of the carcinogenic process in humans" (Weisburger, E. K., Prog. Exp. Tumor Res. 26: 187–201, 1983). During the intervening years new methodologies have been developed. The NTP tumor bioassay system in rats and mice has become the universally accepted standard for rodent toxicity studies. Numerous in vivo non-cancer assays, both chronic and subchronic have been added to the toxicological evaluation including metabolic fate of the chemical, tissue distribution, specific organ toxicity, neurotoxicity, reproductive failure and teratology. A battery of in vitro cytogenetic assays for genotoxicity are routinely incorporated into toxicity evaluation to understand the underlying genetic component in chemically induced carcinogenesis. The growth of molecular genetics has stimulated the NTP to initiate efforts into development of potentially new assays based upon transgenic technology that will extend understanding of the metabolic mechanisms of chemical toxicity down to the level of the gene.

This volume is a complete collection of Technical Report Abstracts through 1992 as taken from the published reports of the National Cancer Institute Bioassay Program (~1971–1978) and it's successor, the National Toxicology Program. Since the Technical Report series now represents over 400 volumes it was felt there was a need to compile a summary of all chronic tumor studies into a single reference volume.

In 1983, the NTP adopted the use of five categories to classify results (see "Levels of Evidence of Carcinogenicity"). Prior to 1983 results were reported as "Positive", "Negative," "Equivocal," and "Inadequate." In 1987, Haseman et al. (Environ. Health Perspect. 74: 229–235, 1987) reclassified these earlier studies (Technical Reports No. 2-200, 202-205) according to the new five category system. We have appended this information to the end of those numbered abstracts enabling a uniformity across the entire compendium of technical reports. Where use information was omitted from earlier abstracts, we have added the major use of the chemical at the time of the study. The report date has also been included at the end of each abstract. There are 22 technical report numbers that were never published, mainly due to inconclusive results. Some of these chemicals have been retested and a technical report published, while others are currently on test. This information is cited at their respective TR numbers in the text. Five technical report numbers were assigned to documents which do not reflect results of chronic tumor studies. One of these reports, TR-108, contains subchronic results for three dyes. Four reports are listed to show the consecutive numbering but abstracts of these documents are not included. They are TR-001 (guidelines establishing standard procedures for toxicology testing in rodents), TR-044 (seminar and workshop proceedings on laboratory procedures), TR-218 (guidelines for quality assurance), and TR-241 (unpublished proceedings of a working seminar on the optimal use of testing facilities).

There are three ways to locate a specific chemical in this compendium. The abstracts appear in this compendium in numerical order by Technical Report. The Alphabetical Index lists the chemicals or reports alphabetically, and the Chemical Abstract Service (CAS) Registry Number Index follows the Chemical Abstract Service Registry (CAS) number notation.

The Numerical Index is a listing of Technical Reports in numerical order and lists CAS Numbers and NTIS document numbers. Full copies of all published technical reports are available through the National Technical Information Service (NTIS) as indicated in the Numerical Index.

Readers may notice differences in editorial styles among the abstracts. It should be noted that these abstracts retain the same style and format as published in the original technical report.

Our appreciation is extended to Amy Noles for her diligence in locating and entering the material contained in this document. Appreciation is also extended to Dr. Tom Goehl for his assistance in chemical identification; to Gloria Nicholson, ISN, for providing programming assistance for the tabular data from the NTP Chemtrack database; to Stan Stasiewicz, Charles Alden, and to many others whose efforts have been instrumental in the preparation of this document.

JAMES K. SELKIRK

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Levels of Evidence of Carcinogenicity

The following information currently appears at the beginning of each Technical Report to inform the reader of the parameters used to classify the results.

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results *(Clear Evidence)* and *(Some Evidence)*; one category for uncertain findings *(Equivocal Evidence)*; one category for no observable effects *(No Evidence)*; and one category for experiments that because of major flaws cannot be evaluated *(Inadequate Study)*.

These categories of interpretative conclusions were first adopted by the National Toxicology Program in June 1983 and then revised in March 1986 for use in the NTP Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. The categories refer to the strength of the experimental evidence and not to either potency or mechanism.

Clear Evidence of Carcinogenic Activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.

Some Evidence of Carcinogenic Activity is demonstrated by studies that are interpreted as showing a chemically-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

Equivocal Evidence of Carcinogenic Activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.

No Evidence of Carcinogenic Activity is demonstrated by studies that are interpreted as showing no chemically-related increases in malignant or benign neoplasms.

Inadequate Study of Carcinogenic Activity is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- The adequacy of the experimental design and conduct
- Occurrence of common versus uncommon neoplasia
- Progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions
- Some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant
- Combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue
- Latency in tumor induction
- Multiplicity in site-specific neoplasia
- Metastases
- Supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species)
- The presence or absence of dose relationships
- The statistical significance of the observed tumor increase
- The concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm
- Survival-adjusted analyses and false positive or false negative concerns
- Structure-activity correlations
- In some cases, genetic toxicology

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ABSTRACTS

TR-1 Guidelines for Carcinogenic Bioassay in Small Rodents

Note to the Reader: These guidelines were published to establish standard procedures for toxicology testing in rodents.

Report Date: February 1976

TR-2 Carcinogenesis Bioassay of Trichloroethylene (CAS No. 79-01-6)

Trichloroethylene (TCE), a halogenated chemical, has been tested for carcinogenicity in the National Cancer Institute's Carcinogenesis Bioassay Program. Trichloroethylene has been used primarily as a solvent in industrial degreasing operations. Other uses have been as a solvent in dry cleaning and food processing, as an ingredient in printing inks, paints. etc., and as a general anesthetic or analgesic.

Industrial grade (>99% pure) trichloroethylene was tested using 50 animals per group at 2 doses and with both sexes of Osborne-Mendel rats and $B6C3F_1$ mice. Twenty of each sex and species were maintained as matched controls, in addition to colony and positive carcinogen controls. Animals were exposed to the compound by oral gavage 5 times per week for 78 weeks. At the end of treatment, animals were observed until terminal sacrifice at 110 weeks for rats and 90 weeks for mice. A complete necropsy and microscopic evaluation of all animals (except 7 of the original 480) was conducted.

Two doses were used with animals started on test at approximately 6 weeks of age. The initial doses used in this test were the estimated maximum tolerated dose (MTD) and 1/2 MTD, as predicted from data obtained in a 6-week toxicity study. For rats, the initial doses were 1,300 and 650 mg/kg body weight. These were changed, based upon survival and body weight data, so that the "time-weighted average" doses were 549 and 1,097 mg/kg for both male and female rats. For mice, the initial doses were 1,000 and 2,000 mg/kg for males and 700 and 1,400 mg/kg for females. The doses were increased so that the "time-weighted average" doses were 1,169 and 2,339 mg/kg for male mice and 869 and 1,739 mg/kg for female mice.

Clinical signs of toxicity, including reduction in weight, were evident in treated rats. These, along with an increased mortality rate necessitated a reduction in doses during the test. In contrast, very little evidence of toxicity was seen in mice, so doses were increased slightly during the study. The increased mortality in treated male mice appears related to the presence of liver tumors.

A variety of neoplastic lesions were observed in rats with no significant difference between trichloroethylene-treated and control animals. The only lesion that might be attributed to the treatment was a chronic nephropathy found in both sexes and at both dose levels.

With both male and female mice, primary malignant tumors of the liver, i.e., hepatocellular carcinoma, were observed in high numbers. For males, 26/50 low dose and 31/48 high dose animals had hepatocellular carcinomas as compared with 1/20 matched controls and 5/77 colony controls. The differences between treated and matched control males at both doses were highly significant ($P<0.01$). For females, hepatocellular carcinomas were observed in 4/50 low dose and 11/47 high dose animals as compared with 0/20 matched controls and 1/80 colony controls. While the difference between the high dose female mice and matched controls was also highly significant ($P<0.01$), the difference at the low dose was less ($P=0.09$). For both male and female mice, age-adjusted tests for linear trend (dose response) were highly significant for hepatocellular carcinoma ($P<0.001$ for males and $P=0.002$ for females).

In male mice at the high doses, hepatocellular carcinomas were observed early in the study. The first was seen at 27 weeks; 9 others were found in male mice dying by the 78th week. The tumor was not observed so early in low dose male or female mice. The diagnosis of hepatocellular carcinoma was based on size, histologic appearance, and presence of metastasis, especially to the lung. No other lesion was significantly elevated ($P<0.05$) in treated mice. The incidence of hepatocellular carcinomas in the trichloroethylene-matched controls was typical of that observed in colony controls.

Carbon tetrachloride (CCl_4) was used as a positive control for the series of chlorinated chemicals which included trichloroethylene. While virtually all male and female mice developed hepatocellular carcinomas following carbon tetrachloride treatment, the response in the Osborne-Mendel rats was considerably less. Only about 5% developed hepatocellular carcinomas. Thus, there appears to be a marked difference in sensitivity to induction of carcinomas by chlorinated compounds between the $B6C3F_1$ mouse and the Osborne-Mendel rat.

The results of this carcinogenesis test of trichloroethylene clearly indicate that trichloroethylene induced a hepatocellular carcinoma response in mice.

While the absence of a similar effect in rats appears most likely attributable to a difference in sensitivity between the Osborne-Mendel rat and the B6C3F$_1$ mouse, the early mortality of rats due to toxicity must also be considered.

Synonyms: trichloroethene; acetylene trichloride; ethinyl trichloride; 1,1,2-trichloroethylene, TCE

Report Date: February 1976

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

Note: Trichloroethylene was subsequently studied by gavage in F344 rats and B6C3F$_1$ mice (See TR-243, reported in 1990) and also in four strains of rats (ACI, August, Marshall, and Osborne-Mendel) by gavage (See TR-273, reported in 1988).

TR-3 Bioassay of 1,1,1-Trichloroethane for Possible Carcinogenicity (CAS No. 71-55-6)

1,1,1-Trichloroethane is one of a group of halogenated hydrocarbons selected for testing in the Carcinogenesis Bioassay Program. The rationale for its selection included its structural relationship to carbon tetrachloride, its wide use in industry, its extensive exposure of humans, and the incomplete knowledge of its carcinogenic potential. In 1959, Browning reported that 1,1,2-trichloroethane was replacing the more toxic industrial solvents: trichloroethylene, tetrachloroethylene, and carbon tetrachloride. The Environmental Protection Agency permits 1,1,1-trichloroethane to be used as a solvent or cosolvent in pesticide formulations for the postharvest fumigation of citrus fruits.

The carcinogenesis bioassay of technical grade 1,1,1-trichloroethane was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. 1,1,1-Trichloroethane was administered orally by gavage in corn oil to 50 animals of each sex and species at two dose levels 5 days per week for 78 weeks.

Rats: The experiment was originally started using doses of 3,000 and 1,500 mg/kg of body weight. After a few weeks the study was terminated, and the animals discarded because of marked signs of intoxication. The experiment was restarted with rats 7 weeks of age that were put on doses of 1,500 and 750 mg/kg. There was a moderate depression of body weight in the first year of the study. During the second year a yellow discoloration of the fur of the lower abdomen and increased eye and nasal discharge and dyspnea were noted. Both males and females given the test chemical exhibited early mortality when compared with the untreated controls, and the statistical test for dose-related trend was significant ($P<0.04$). All surviving animals were killed at 117 weeks of age.

Mice: Male and female weanlings were started on test at 5 weeks of age and killed at 96 weeks of age. Initially, the doses for male and female mice were 4,000 and 2,000 mg/kg body weight. During the 10th week of the study, doses were increased to 5,000 and 2,500 mg/kg, since the animals apparently could tolerate a higher dose. Doses were again increased at week 20 to 6,000 and 3,000 mg/kg and maintained at these levels to the end of the study. Time-weighted average doses for the high- and low-dose mice were, respectively, 5,615 and 2,807 mg/kg. There was a moderate depression of body weight throughout the study in both sexes of mice, and the survival was significantly decreased. In the female mice, there was a positive dose-related trend ($P=0.002$) in the proportions surviving.

A variety of neoplasms were represented in both 1,1,1-trichloroethane-treated and matched-control rats and mice. However, each type of neoplasm has been encountered previously as a lesion in untreated rats or mice. The neoplasms observed are not believed attributable to 1,1,1-trichloroethane exposure, since no relationship was established between the dosage groups, the species, sex, type of neoplasm, or the site of occurrence. Even if such a relationship were inferred, it would be inappropriate to make an assessment of carcinogenicity of 1,1,1-trichloroethane on the basis of this test, because of the abbreviated life spans of both the rats and the mice.

Synonym: methylchloroform

Report Date: January 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Inadequate Study
Female Mice:	Inadequate Study

TR-4 Bioassay of Dimethoate for Possible Carcinogenicity (CAS No. 60-51-5)

Dimethoate is the common name for the organophosphorous insecticide, o,o-dimethyl-S-(N-methylcarbamoyl-methyl)phosphorodithioate. This compound, which has been in use since 1956 as an insecticide and acaricide, is registered for insect and mite control on agricultural crops and ornamental plants. It is also registered as a residual fly spray in animal quarters.

A bioassay of the carcinogenicity of technical-grade dimethoate was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. The test material was administered in feed to groups of 50 rats of each sex at either of two concentrations for 80 weeks, followed by 35 weeks of observation. Initial doses were not well tolerated; therefore, they were reduced during the study. The "time-weighted average doses" for rats were 155 and 310 ppm for males and 192 and 384 ppm for females. All surviving rats were killed between 113 and 115 weeks.

Dimethoate was administered in feed to groups of 50 male and 50 female mice at two concentrations. Female mice received diets containing 200 and 500 ppm of dimethoate for 80 weeks; male mice received the same

dosage. However, high-dose males were returned to the control diet at 60 weeks, and low-dose males at 69 weeks. All surviving mice were killed between 93 and 94 weeks.

Tremors and hyperexcitability, both indications of dimethoate toxicity, were observed in the treated animals. However, it is considered that the low-dose group of rats and both dose groups of mice survived long enough to permit an evaluation of carcinogenicity. Pathologic evaluation revealed no statistically significant increase in tumors associated with dimethoate treatment in either species of animal, and it is concluded that there was no carcinogenic effect under the conditions of the experiment.

Synonyms: o,o-dimethyl-S-(N-methylcarbamoyl-methyl) phosphorodithioate

Report Date: January 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-5 Bioassay of Proflavine for Possible Carcinogenicity (CAS No. 952-23-8)

Proflavine is a synthetic acridine dye which early in this century was found to have bacteriostatic and bacteriocidal properties when administered topically. During World War II it was widely used as a would antiseptic. With the advent of more specific and less toxic antibiotics, its clinical importance declined, until it was reintroduced recently in combination with ultraviolet light for the treatment of psoriasis and type-II herpesvirus infection.

A bioassay of the carcinogenicity of proflavine monohydrochloride hemihydrate was conducted using Fischer 344/CR rats and B6C3F_1 mice. The compound was administered in the diet at concentrations of 300 and 600 ppm to groups of 50 rats for 109 weeks and at concentrations of 200 and 400 ppm to groups of 50 mice for 104 weeks. The animals were subjected to necropsy and histopathologic evaluation as they died or at the end of their periods of treatment.

Average weights attained by high-dose groups were consistently lower than those of control groups; weights of low-dose groups showed essentially no differences from those of the controls. Survival rates of the treated rats and mice did not differ from those of the controls except for a lower rate among the female mice.

Five malignant neoplasms of the intestinal tract consisting of three leiomyosarcomas of the small intestine, a sarcoma near the colon area, and an adenocarcinoma of the small intestine were observed in five of the high-dose male rats. None were observed in other treatment or control groups. If these five intestinal neoplasms are considered together, they are significant at the $P = 0.026$ level using the Fisher exact test. A positive dose-related trend ($P = 0.034$) was also present for the three leiomyosarcomas.

The observed incidence of hepatocellular carcinoma in female mice was 4/50 (8%) in the control group, 20/49 (41%) in the low-dose group, and 22/50 (44%) in the high-dose group. The test for dose-related trend showed a level of significance of $P < 0.001$. In male mice, the observed incidence of hepatocellular carcinoma was 20/49 (41%) in the control group 28/49 (57%) in the low-dose group, and 30/50 (60%) in the high-dose group. The dose-related trend was significant at $P = 0.057$, and the high dose was significant at $P = 0.044$.

The unusually high incidence of hepatocellular carcinomas and hemangiosarcomas in control male mice and the unusually high incidence of malignant lymphomas in all groups of female mice in conjunction with the fact that a positive-control carcinogen was tested in the same room with these animals, raises a question of the validity of these bioassay results.

Synonym: 3,6-diaminoacridine

Report Date: January 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Equivocal

TR-6 Bioassays of Nitrilotriacetic Acid (NTA) and Nitrilotriacetic Acid, Trisodium Salt, Monohydrate ($Na_3NTA·H_2O$) for Possible Carcinogenicity (CAS No. 139-13-9) (NTA) (CAS No. 18662-53-8) ($Na_3NTA·H_2O$)

Nitrilotriacetic acid (NTA) is a synthetic aminopolycarboxylic acid chelating agent used chiefly as a replacement for phosphates in detergents. NTA sequesters magnesium and calcium ions present in hard water, which would normally inhibit the activity of detergent surfactants. In December 1970, the detergent industry voluntarily suspended such applications of NTA in the United States following an unpublished government report indicating that the compound was teratogenic. During that year the annual production of NTA was 150 million pounds, of which 86-92% was used in detergents. Major nondetergent uses, for which NTA is still being produced, include water treatment, textile treatment, metal plating and cleaning, and pulp and paper processing. To a lesser extent, NTA is used in leather tanning, photographic development, synthetic rubber production, the manufacture of pharmaceuticals, agriculture (in herbicide formations and micronutrient solutions), and in the separation of rare-earth elements.

Bioassays for the carcinogenicity of nitrilotriacetic acid, trisodium salt, monohydrate ($Na_3NTA·H_2O$) were conducted at Stanford Research Institute (SRI), using Fischer 344 rats and at Litton Bionetics, Inc. (LBI), using both Fischer 344 rats, and B6C3F_1 mice. Similar bioassays using rats and mice, were conducted at LBI on the

free acid, nitrilotriacetic acid (NTA). Each chemical was mixed in respective diets and administered *ad libitum*. The $Na_3NTA \cdot H_2O$ was tested in rats at SRI at 200, 2,000, and 20,000 ppm for a 24-month period. It was also tested in rats at LBI at 7,500 and 15,000 ppm and in mice at 2,500 and 5,000 ppm using 18-month feeding periods for both species. The NTA was tested in rats and mice at LBI at 7,500 and 15,000 ppm for the 18-month period. The numbers of animals used in tests at SRI were 24 of each sex for each dose group and for the controls; at LBI, 50 of each sex for each dose group and 20 of each sex for the controls. Since equimolar quantities of $Na_3NTA \cdot H_2O$ and NTA were not used, given concentrations of $Na_3NTA \cdot H_2O$ represented 30% less NTA than did equal concentrations of the free acid.

Average weights attained by high-dose groups of rats and mice were consistently lower than those of control groups. Less difference was observed with the low-dose groups. Survival, however, was not decreased by the compounds administered, except in rats given 20,000 ppm $Na_3NTA \cdot H_2O$.

Lesions of the urinary tract were found in most treated groups of both rats and mice. They were characterized, especially in the high-dose groups, by primary tumors of epithelial origin. These tumors were particularly significant since they were not found in the urinary tract of the control mice and only rarely occur spontaneously in the strains of animals on test. Lesions of the urinary tract were also characterized by hydronephrosis and/or nephritis in high-dose rats and by nephritis in both high- and low-dose mice.

Statistical evidence of the carcinogenicity of $Na_3NTA \cdot H_2O$ and NTA was provided by incidences of tumors at different sites in the urinary tract. For example, among animals given 20,000 ppm $Na_3NTA \cdot H_2O$ at SRI, tumors of the kidney occurred in male (treated, 9/24; untreated, 0/24; $P = 0.001$) and female (treated, 4/24; untreated, 0/24; $P = 0.054$) rats; tumors of the ureter in male (treated, 8/24; untreated, 0/24; $P = 0.002$) and female (treated, 6/24; untreated, 0/24; $P = 0.011$) rats; and tumors of the bladder, in female rats (treated, 5/24; untreated, 0/22; $P = 0.031$). Similarly, among animals given 15,000 ppm NTA at LBI, tumors of the bladder occurred in female rats (treated, 12/48; untreated, 0/18; $P = 0.014$) and tumors of the kidney occurred in male mice (treated, 24/44; untreated, 0/20; $P < 0.001$). Additional tests at LBI, using 15,000 and 7,500 ppm $Na_3NTA \cdot H_2O$ and 7,500 ppm NTA in male and female rats, 15,000 ppm NTA in female mice, and 7,500 ppm NTA in male mice, also induced tumors of the urinary tract, but in numbers too low to be statistically significant. Metastatic tumors, appearing to have arisen from primary tumors of the urinary tract, were found in 5/24 male and 5/24 female rats given 20,000 ppm $Na_3NTA \cdot H_2O$ at SRI and in one male rat given 15,000 ppm NTA at LBI; none were found in rats given lower doses or in mice.

Thus, NTA and $Na_3NTA \cdot H_2O$ were shown to be carcinogenic to the urinary tracts of both rats and mice at the higher doses tested. Lower doses, as delineated in this report, did not induce significant numbers of such lesions.

Report Date: January 1977

Levels of Evidence of Carcinogenicity:

For Nitrilotriacetic Acid (NTA) at LBI:
Male Rats: Positive
Female Rats: Positive
Male Mice: Positive
Female Mice: Positive

For Nitrilotriacetic Acid Trisodium Monohydrate at SRI:
Male Rats: Positive
Female Rats: Positive

For Nitrilotriacetic Acid Trisodium Monohydrate at LBI:
Male Rats: Equivocal
Female Rats: Equivocal
Male Mice: Negative
Female Mice: Negative

TR-7 Bioassay of Phenformin for Possible Carcinogenicity (CAS No. 114-86-3)*

Phenformin is a synthetic oral hypoglycemic agent used to control maturity-onset diabetes. Pharmacologically, phenformin acts to enhance anaerobic glycolysis, decrease gluconeogenesis, and inhibit intestinal absorption of glucose. This compound was selected for carcinogenicity testing since, in the treatment of diabetes, it is administered chronically.

A bioassay of the carcinogenicity of phenformin hydrochloride was conducted using Fischer 344 rats and $B6C3F_1$ mice. The compound was administered in the diet for 78 weeks to groups of 35 animals of each species and sex, using concentrations of 15,000 and 30,000 ppm for rats and concentrations of 1,200 and 2,500 ppm for mice. Treatment was followed by a period of observation of 26 weeks. Control groups consisted of 15 untreated animals of each species and sex.

Average weights attained by treated groups of rats and mice were consistently lower than those of control groups in all tests except that for male rats, in which case the weights shown by treated and control animals were indistinguishable. Survival was apparently unaffected in both species by treatment with phenformin, but was poor in mice due to intercurrent disease.

Tumors appearing in treated rats and mice were similar in type and number to those in controls, and no pathologic or statistical evidence of induction of tumors in these species by phenformin was found.

Synonym: 1-phenethylbiguanide hydrochloride

Report Date: January 1977

Levels of Evidence of Carcinogenicity:

Male Rats: Negative
Female Rats: Negative
Male Mice: Negative
Female Mice: Negative

*The technical report states that Phenformin Hydrochloride (CAS No. 834-28-6) was the actual chemical tested rather than Phenformin in its pure form; therefore, the CAS number for Phenformin Hydrochloride is used to track this study in the NTP CHEMTRACK database.

TR-8 Bioassay of Chlordane for Possible Carcinogenicity (CAS No. 57-74-9)

Chlordane is a member of the cyclodiene group of chlorinated insecticides, which includes aldrin, dieldrin, endrin, heptachlor, and endosulfan. It was introduced in 1945 and was the first chlorinated cyclodiene developed for insect control. It is effective on a wide variety of insects of agricultural, industrial, and domestic importance. The compound was registered for use on more than 40 vegetable and 27 fruit crops. About a third of the amount used in the United States is applied to pests of the home, garden, lawn, and turf.

A bioassay of analytical-grade chlordane for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and $B6C3F_1$ mice. Groups of 50 rats of each sex were administered low or high concentrations of the chlordane for 80 weeks, then observed for 29 weeks. Because of toxic effects, doses were reduced for both male and female rats during the course of the tests. Time-weighted average doses used for the male rats were 203.5 and 407.0 ppm; for the females, 120.8 and 241.5 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups combined with 50 untreated male and 50 untreated female rats from similar bioassays of five other compounds. All surviving rats were killed at 109 weeks.

Groups of 50 mice of each sex were administered the test material at low or high concentrations for 80 weeks, then observed for 10 weeks. The low- and high-dose groups were tested at different calendar times, but each of the treated groups was tested along with a concurrent control. Because of toxic effects, doses were reduced for female mice during the course of the tests; however, it was possible to increase the doses for the male mice. The time-weighted average doses used for the male mice were 29.9 and 56.2 ppm; for the females, 30.1 and 63.8 ppm. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups combined with 70 untreated male and 80 untreated female mice from similar bioassays of five other compounds. All surviving mice were killed at 90-91 weeks.

The effects of chlordane on body weights and other clinical signs in rats and mice indicated that the dosages used were near the maximum permissible. This was evident in that the average body weights of the high-dose male and female rats were consistently lower than those of the untreated controls, while differences between the low-dose and control rats were negligible. Body weights of mice given either low or high doses showed little or no effect of the chlordane; however, other adverse clinical signs were seen with greater frequency than in control mice.

The effects of chlordane on survival rates indicated that mortality was dose-related for female rats and for male mice. However, a substantial proportion of most groups of animals survived to an age at which tumors could be expected to appear; male control rats, for unknown reasons, showed an abnormally low survival rate.

Hepatocellular carcinoma showed a highly significant dose-related trend for mice, using either matched controls (for males, controls 2/18, low dose 16/48, high dose 43/49, $P<0.0001$; for females, controls 0/19, low dose 3/47, high dose 34/49, $P<0.0001$) or pooled controls (for males, controls 17/92, $P<0.0001$; for females, controls 3/78, $P<0.0001$). These high levels of significance were maintained when hepatocellular carcinoma was combined with nodular hyperplasia or when the data were subjected to life-table adjustment. No other tumors were found in mice in sufficient numbers to justify analysis.

In contrast to findings with mice, hepatocellular carcinoma failed to appear at a significant rate of incidence in rats administered chlordane. Further, the number of lesions of the liver in rats did not become significant with the addition of nodular neoplasia or with the application of life-table adjustment to the data.

There was significant statistical evidence for the induction in treated male rats of proliferative lesions of follicular cells of the thyroid and of malignant fibrous histiocytoma, but these findings were discounted because the rates of incidence were comparatively low and/or are known to be variable in control rat populations.

It is concluded that under the conditions of this bioassay chlordane is carcinogenic for the liver in mice.

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-9 Bioassay of Heptachlor for Possible Carcinogenicity (CAS No. 76-44-8)

Heptachlor is a member of the cyclodiene group of chlorinated insecticides (aldrin, dieldrin, endrin, chlordane, heptachlor, and endosulfan) that was developed in the 15 years following World War II. It was registered as a commercial pesticide in 1952 for foliar, soil, and structure applications and for malarial control programs; after 1960 it was used primarily in soil applications against agricultural pests and to a lesser extent against termites.

A bioassay of technical-grade heptachlor for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and $B6C3F_1$ mice. Groups of 50 rats of each sex were administered low or high concentrations of the heptachlor for 80 weeks, then observed for 30 weeks. Doses for females were first increased, but because of toxic effects the doses were then reduced twice for both male and female rats during the

remaining course of the tests. Time-weighted average doses used for the male rats were 38.9 and 77.9 ppm; for the females, 25.7 and 51.3 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups combined with 50 untreated male and 50 untreated female rats from similar bioassays of five other compounds. All surviving rats were killed at 110-111 weeks.

Groups of 50 mice of each sex were administered the test material at low or high concentrations for 80 weeks, then observed for 10 weeks. The low- and high-dose groups were tested at different calendar times, but a concurrent control group was started with each. Because of toxic effects, doses were reduced once for the males at 17-18 weeks after the initiation of tests; twice for the females, at 17 and 30 weeks, after the initiation of tests. The time-weighted average doses used for the male mice were 6.1 and 13.8 ppm; for the females, 9 and 18 ppm. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups combined with 90 untreated male and 70 untreated female mice from similar bioassays of five other compounds. All surviving mice were killed at 90-91 weeks.

The effects of heptachlor on body weights and other clinical signs in rats and mice indicated that the dosages used were near the maximum permissible. This was evident in that average body weights of rats treated with high doses were consistently lower than those of untreated controls, while body weights of low-dose rats were unaffected. Body weights of mice given either high or low doses showed little or no differences from those of control mice; however, other adverse clinical signs were found in high-dose mice, predominantly in the females.

The effects of heptachlor on survival rates indicated that mortality was dose-related for both female rats and female mice, but not for males of either species. However, a substantial proportion of all groups of animals survived to an age at which tumors could be expected to appear.

In mice, hepatocellular carcinoma showed a highly significant dose-related trend in both males (matched controls 5/19, low dose 11/46, high dose 34/47, $P = 0.001$) and females (control 2/10, low dose 3/47, high dose 30/42, $P < 0.0001$). When pooled controls were used for the comparison, the significance of the trend in males increased to $P < 0.0001$. Comparably high levels of significance were attained when the data were subjected to life-table adjustment. No other tumors were found in mice in sufficient numbers to justify analysis.

In marked contrast to the findings observed in mice, no hepatic tumors were observed in rats administered heptachlor. There was significant statistical evidence for the induction of proliferative lesions of follicular cells of the thyroid in treated female rats, but this finding was discounted because the rates of incidence were comparatively low and are known to be variable in control rat populations.

It is concluded that under the conditions of this bioassay, heptachlor is carcinogenic for the liver in mice.

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Equivocal
Male Mice:	Positive
Female Mice:	Positive

TR-10 Bioassay of Dichlorvos for Possible Carcinogenicity (CAS No. 62-73-7)

Dichlorvos is an organophosphate insecticide with contact and vapor action. It has been used widely for control of agricultural, industrial, and domestic pests since the 1950's. Dichlorvos is available in oil solutions, emulsifiable concentrations, and aerosol formulations; the impregnation of dichlorvos in a polyvinyl chloride base (pellets, strips, blocks, etc.) for delayed release is a widely used method for the control of pests in domestic and industrial situations.

A bioassay for the possible carcinogenicity of technical-grade dichlorvos was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. The test material was administered in the diet at two concentrations for 80 weeks to groups of 50 animals of each species and sex. The test animals were held for observation, and surviving rats were killed at 110-111 weeks and surviving mice at 92-94 weeks from initiation of the study. Initial doses in both species were not well tolerated and they were lowered after a few weeks. Time-weighted average doses for both males and females were 150 and 326 ppm for rats and 318 and 635 ppm for mice. The matched controls consisted of 10 rats of each sex and 10 mice of each sex; the pooled controls consisted of 60 rats of each sex, 100 male mice, and 80 female mice. All surviving rats were killed at 106 to 109 weeks; surviving mice, at 92 to 94 weeks.

After the doses were reduced, no toxic signs directly attributable to the compound were observed. However, average weights of high-dose animals were slightly depressed. Survival was not dose-related in either species. Microscopic study of the tissues of treated animals and matched and pooled controls revealed no statistically significant increase in the incidence of tumors attributable to exposure to dichlorvos in either animal species. The significance of the three esophageal tumors in male and female mice and of malignant fibrous histiocytomas in male mice is unclear and there is insufficient evidence to indicate they were associated with dichlorvos treatment. Thus under the conditions of this study, dichlorvos was not demonstrated to be carcinogenic.

Synonym: 2,2-dichlorovinyl dimethylphosphate

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

Note: Dichlorvos was subsequently studied by gavage in F344 rats and $B6C3F_1$ mice (See TR-342, reported 1989).

TR-11 Bioassay of Trisodium Ethylenediaminetetraacetate Trihydrate (EDTA) for Possible Carcinogenicity (CAS No. 150-38-9)

A bioassay of the chelating agent, trisodium ethylenediaminetetraacetate trihydrate ($Na_3EDTA{\cdot}3H_2O$), for possible carcinogenicity was conducted by administering the test material in feed to Fischer 344 rats and $B6C3F_1$ mice. The chemical was administered to 50 males and 50 females of each species at low and high concentrations, 3,750 and 7,500 ppm, for 103 weeks. Matched-control groups were composed of 20 males and 20 females of each species.

No compound-related signs of clinical toxicity were noted. Although a variety of tumors occurred among test and control animals of both species, no tumors were related to treatment. Since survival was satisfactory and showed no consistent variation among test and control groups, the absence of treatment-related tumors could not be attributed to early mortality.

Synonym: EDTA

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-12 Bioassay of Endrin for Possible Carcinogenicity (CAS No. 72-20-8)

Endrin is an organochlorine pesticide having a structural characteristic of the cyclodiene group, which includes aldrin (CAS No. 309-00-2), dieldrin (CAS No. 60-57-1), chlordane (CAS No. 57-74-9), heptachlor (CAS No. 76-44-8), and endosulfan (CAS No. 115-29-7). It is the most acutely toxic compound in the cyclodiene group but is less persistent in the environment than DDT or dieldrin. As an insecticide, it is currently used for small grains, sugarcane, and cotton; as an avicide, for forest seed and perch applications; and as a rodenticide, for forest seed and orchard soil applications.

A bioassay of technical-grade endrin for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice. Groups of 50 rats of each sex were administered one of two doses of endrin for 80 weeks, then observed for 31 or 34 weeks. The doses used for the male rats were 2.5 or 5 ppm. The initial doses of 5 or 10 ppm used for the females were not well tolerated and were reduced during the study. The time-weighted average doses used for the females were 3 or 6 ppm. Matched controls consisted of groups of 10 rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 40 untreated male and 40 untreated female rats from similar bioassays of other test chemicals. All surviving rats were killed at 110 to 114 weeks.

Groups of 50 mice of each sex were administered endrin at one of two doses for 80 weeks, then observed for 10 or 11 weeks. Initial doses of 2.5 or 5 ppm used for the males were not well tolerated and were reduced during the study. The time-weighted average doses used for the males were 1.6 or 3.2 ppm; the doses used for the females were 2.5 or 5 ppm. Matched controls consisted of groups of 10 mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 50 untreated male and 50 untreated female mice from similar bioassays of other test chemicals. All surviving mice were killed at 90 or 91 weeks.

The clinical signs observed in both rats and mice indicated that the doses of endrin used were near the maximum tolerated doses. In mice these signs included hyperexcitability, a manifestation of toxicity of the organochlorine pesticides. However, mean body weights of the rats and mice were not affected by administration of endrin.

Although the survival of the high-dose male mice at the end of the study was markedly lower than that of the controls, the survivals of the low- and high-dose female mice and male and female rats were unaffected by the endrin. The survival of the low-dose male mice could not be evaluated, due to the accidental administration of excessive quantities of endrin to this group during week 66. However, a substantial portion of all groups of rats and mice survived to an age at which tumors could be expected to occur.

In rats, the combination of adenomas and carcinomas of the adrenal occurred at the following incidences – males: pooled controls 2/44, matched controls 2/9, low-dose 4/46, high-dose 8/44; females: pooled controls 4/46, matched controls 3/9, low-dose 16/49, high-dose 7/47. These incidences did not show consistent statistical significance. Furthermore, the incidences of the tumors in the matched controls of either sex were higher than those of the corresponding pooled controls, and the incidences in the matched controls equaled or exceeded those in any of the respective dosed groups. Thus, these tumors cannot be clearly related to administration of the test chemical.

In mice, no tumors occurred in dosed groups at incidences that were significantly higher than those in pooled or matched controls.

It is concluded that under the conditions of this bioassay, endrin was not carcinogenic for Osborne-Mendel rats or for $B6C3F_1$ mice.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-13 Bioassay of Tetrachloroethylene for Possible Carcinogenicity (CAS No. 127-18-4)

Tetrachloroethylene is one of a group of halogenated organic solvents selected by the National Cancer Institute (NCI) for inclusion in the Carcinogenesis Bioassay Program. These solvents were selected on the basis of large-scale production, extensive use, and lack of adequate chronic toxicity data.

The bioassay of U.S.P.-grade tetrachloroethylene for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Tetrachloroethylene in corn oil was administered by gavage at either of two dosages to groups of 50 male and 50 female animals of each species, 5 days a week, over a period of 78 weeks followed by an observation period of 32 weeks for rats and 12 weeks for mice.

Initial dosage levels for the chronic bioassay were selected on the basis of a preliminary subchronic toxicity test. Subsequent dosage adjustments were made during the course of the chronic bioassay. The high and low time-weighted average dosages of tetrachloroethylene in the chronic study were 941 and 471 mg/kg/day for the male rats, 949 and 474 mg/kg/day for the female rats, 1,072 and 536 mg/kg/day for the male mice, and 772 and 386 mg/kg/day for the female mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil at the same time that dosed animals were gavaged with tetrachloroethylene mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals received no gavage treatments.

No significant increased incidence of neoplastic lesions was observed in treated rats. In both dosed and control rats, respiratory disease was observed with increasing frequency for the latter part of the first year until termination of the bioassay. Lesions indicative of pneumonia were observed in nearly all rats at necropsy. A high incidence of toxic nephropathy was observed in treated rats. Toxic nephropathy was noted in rats that died early in the study (as early as week 20 for male rats and week 28 for female rats). Mortality of rats was dose-related. Fifty percent of the high dose males had died by week 44 and 50 percent of the high dose females had died by week 66.

In both male and female mice, administration of tetrachloroethylene was associated with a significantly increased incidence of hepatocellular carcinoma. Hepatocellular carcinomas were observed in 2/17 (12 percent) untreated control males, 2/20 (10 percent) vehicle control males, 32/49 (65 percent) low dose males, 27/48 (56 percent) high dose males, 2/20 (10 percent) untreated control females, 0/20 vehicle control females, 19/48 (40 percent) low dose females, and 19/48 (40 percent) high dose females. Hepatocellular carcinomas metastasized to the kidney in one untreated control male and to the lung in three low dose males, one low dose female, and one high dose female. Toxic nephropathy, similar to that observed in rats, was also observed in treated but not control mice.

Fisher exact tests indicated a highly significant increased incidence of hepatocellular carcinoma for each dosed group compared to each control group. Cochran-Armitage tests showed a highly significant positive association between increased dosage and elevated tumor incidence. Time-adjusted analyses, based on Kaplan and Meier survival curves, indicated that the estimated probability of observing hepatocellular carcinoma by week 91 was 1.00 in a dosed male mouse and 0.938 in a dosed female mouse.

The results of the bioassay of tetrachloroethylene in Osborne-Mendel rats do not allow an evaluation of the carcinogenicity of this compound due to the high rate of early death among the treated animals. However, under the condition of this study, tetrachloroethylene is a liver carcinogen in $B6C3F_1$ mice of both sexes.

Synonyms: perchloroethylene; carbon dichloride

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Positive
Female Mice:	Positive

Note: Tetrachloroethylene was subsequently studied by inhalation in F344 rats and $B6C3F_1$ mice (See TR-311, reported 1986).

TR-14 Bioassay of Lindane for Possible Carcinogenicity (CAS No. 58-89-9)

Lindane is an organochlorine pesticide that is registered for use in soil, foliar, and seed treatment for a large variety of fruit and vegetable crops, and for use on livestock, pets, and agricultural premises. Residues of lindane may be persistent in soil and foods. There may also be direct human exposure to lindane through its use in pharmaceutical preparation or in public health pest control.

A bioassay for possible carcinogenicity of lindane was conducted by administering the test chemical in the diet to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered lindane at one of two doses for 80 weeks, then observed for 29-30 weeks. Time-weighted average doses for males were 236 or 472 ppm; those for females were 135 or 270 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 45 untreated male and 45 untreated female rats from similar bioassays of four other test chemicals. All surviving rats were killed at 108-110 weeks.

Groups of 50 mice of each sex were administered lindane at one of two doses, either 80 or 160 ppm, for 80 weeks, then observed for an additional 10-11 weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 40 untreated

male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 90-91 weeks.

Neither the mean body weights of rats nor those of mice showed consistent effects from the administration of lindane. The physical condition of the surviving treated mice deteriorated during the final 6 weeks on study. Except for the female matched-control group of rats, survival of all groups of rats and mice was adequate for meaningful statistical analyses of the incidence of tumors.

In rats, no tumors occurred at a statistically significant incidence in the treated groups of either sex.

In mice, the incidence of hepatocellular carcinoma in low-dose males was significant when compared with that in the pooled controls (controls 5/49, low-dose 19/49, P = 0.001). This finding, by itself, is insufficient to establish the carcinogenicity of lindane. The incidence of hepatocellular carcinoma in high-dose male mice (9/46) was not significantly different from that in the matched (2/10) or pooled controls.

It is concluded that under the conditions of this bioassay, lindane was not carcinogenic for Osborne-Mendel rats or $B6C3F_1$ mice.

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Netative

TR-15 Bioassay of Captan for Possible Carcinogenicity (CAS No. 133-06-2)

Captan is a broad-spectrum fungicide which inhibits mycelial growth from germinating fungus spores. As a result, it has effective protection action, although it will not eradicate a preexisting infection. Because captan is a nonpersistent fungicide, directions for use indicate that it should be reapplied every week as necessary to maintain control. It has been one of the most widely used fungicides since its introduction in 1950.

A bioassay of technical-grade captan for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered one of two doses of captan for 80 weeks, then observed for 33 or 34 weeks. The time-weighted average doses for both sexes of rats were 2,525 or 6,050 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 75 untreated male and 75 untreated female rats from similar bioassays of six other test chemicals. All surviving rats were killed at 113-114 weeks.

Groups of 50 mice of each sex were administered the test material at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 11 weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 80 untreated male and 80 untreated female mice from similar bioassays of six other test chemicals. All surviving mice were killed at 90-91 weeks.

The mean body weights of both low- and high-dose rats and high-dose mice were lower than those of the matched controls throughout most of the study. Mortality rates did not show statistically significant dose-related trends in either sex of either species.

In rats, a positive dose-related trend and a difference between incidences of tumors in high-dose and pooled-control groups were found in females when the data for adrenal cortical adenoma were combined with those for adrenal cortical carcinoma (pooled controls, 0/64, low-dose 2/50, high-dose 3/47, P = 0.047). There was also a positive dose-related trend for the incidence of C-cell adenoma of the thyroid in female rats (pooled controls 1/66, low-dose 1/49, high-dose 4/44, P = 0.035). These endocrine tumors in female rats are believed to have been spontaneous, and not related to treatment.

In mice, the incidences of polypoid carcinoma (adenocarcinoma in adenomatous polyp) of the duodenum were statistically significant using tests for a positive dose-related trend both in male mice (pooled controls 0/68, low-dose 1/43, high-dose 3/46, P = 0.033) and in female mice (pooled controls 0/68, low-dose 0/49, high-dose 3/48, P = 0.022). When the incidences of adenomatous polyp, NOS (not otherwise specified), were combined with those of polypoid carcinoma for statistical analysis, the tests for male mice indicated a substantial increase in significance (pooled controls 0/68, low-dose 3/43, high-dose 5/46, P = 0.008).

It is concluded that under the conditions of this bioassay, tumors in the duodenum of $B6C3F_1$ mice were associated with treatment with captan, but there was no convincing evidence that the tumors observed in Osborne-Mendel rats were related to treatment.

Synonym: N-((trichloromethyl)thio)-4-cyclohexene-1,2-dicarboximide

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-16 Bioassay of Phosphamidon for Possible Carcinogenicity (CAS No. 13171-21-6)

Phosphamidon is an organophosphorus compound used as a broad-spectrum insecticide in agriculture since 1956. It is toxic both systemically and by contact, and acts through the inhibition of cholinesterase. Phosphamidon is currently registered for use by both ground and aerial

applications on vegetables, fruits and field crops with tolerances for residues from 0.1 to 1.0 ppm.

A bioassay of technical-grade phosphamidon for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. The test material was administered in feed to 50 rats and 50 mice of each sex at one of two doses, either 80 or 160 ppm. The rats were fed the test chemical for 80 weeks, then observed without compound administration for 30 or 31 weeks; the low-dose male mice were fed for 71 weeks, then observed for 19 weeks; the high-dose male mice were fed for 62 weeks, then observed for 28 weeks; and the low- and high-dose female mice were fed for 80 weeks, then observed for 10 or 11 weeks. Matched controls consisted of groups of 10 untreated rats or 10 untreated mice of each sex; pooled controls consisted of the matched controls combined with 85 male and 85 female untreated rats or 80 male and 80 female untreated mice from similar bioassays of eight other test chemicals. All surviving rats were killed at 110 or 111 weeks; all surviving mice were killed at 90 or 91 weeks.

Hyperexcitability and tremors, both indications of phosphamidon toxicity, were observed in dosed rats and mice. However, sufficient numbers of all groups of both species were at risk for the development of late-appearing tumors.

In male rats, the combined incidence of hemangiomas and hemangiosarcomas in the spleen showed a statistically significant (P = 0.012) dose-related trend. However, the comparison with matched controls was not significant, and the historical records of this laboratory on untreated males of this strain show a tumor incidence of 6/240 (3%) with incidences in individual control groups as high as 3/9 (33%) and 2/9 (22%), compared with 5/49 (10%) seen in the high-dose group in this study. No hemangiomas or hemangiosarcomas were found in the females.

In female rats, the Cochran-Armitage test for dose-related trend was significant (P = 0.003) for C-cell adenomas and carcinomas of the thyroid when pooled controls were compared with the dosed groups. The incidences of these tumors were also significant when low-dose females (P = 0.003) and high-dose females (P = 0.004) were compared directly with pooled controls. However, the historical records of this laboratory show a tumor incidence of 16/235 (7%) in untreated female rats of this strain of female rats, with incidences in individual control groups as high as 3/9 (33%) and 3/10 (30%); these data are therefore considered marginal and insufficient to establish an association between the tumors and administration of the chemical. In males, the incidence of these tumors was not statistically significant.

In mice, no tumor occurred at a higher incidence in dosed animals than in controls.

It is concluded that under the conditions of this bioassay, technical-grade phosphamidon was not carcinogenic for B6C3F$_1$ mice. The data obtained in this bioassay with Osborne-Mendel rats are insufficient to allow the interpretation that technical-grade phosphamidon is carcinogenic in this species.

Synonym: dimethyl 2-chloro-2-diethyl-carbamoyl-1-methylvinyl phosphate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Negative
Female Mice:	Negative

TR-17 Bioassay of Photodieldrin for Possible Carcinogenicity (CAS No. 13366-73-9)

Photodieldrin is a photochemical conversion product of dieldrin. Although it has never been produced commercially, photodieldrin was selected for testing in 1969 because it was a photochemical conversion product of dieldrin. At that time dieldrin was used extensively as a pesticide.

A bioassay of dieldrin-free photodieldrin (synthesized by Gulf South Research Institute) for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and B6C3F$_1$ mice.

Groups of 50 rats of each sex were initially administered photodieldrin at one of two doses, either 5 or 10 ppm. Because of neurotoxic signs, doses in the females were reduced after 30 weeks. Total periods of treatment for low- and high-doge males and low-dose females were 80 weeks, followed by periods of 31 or 32 weeks of additional observation; the total period of treatment for the high-dose females was 59 weeks, followed by a period of additional observation of 53 weeks. The time-weighted average doses for the females were 3.4 or 7.5 ppm. Matched controls consisted of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 65 untreated male and 65 untreated female rats from similarly performed bioassays of six other test chemicals. All surviving rats were killed at 111-112 weeks.

Groups of 50 mice of each sex were administered photodieldrin at one of two doses, either 0.32 or 0.64 ppm, for 80 weeks, then observed for an additional 13 weeks. Matched controls consisted of groups of 10 untreated mice of each sex at each dose; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 60 untreated male and 60 untreated female mice from similarly performed bioassays of six other test chemicals. All surviving mice were killed at 93 weeks.

Mean body weights attained by low- and high dose male and female rats and mice were essentially unaffected by photodieldrin. Convulsions and hyperactivity were noted in treated male and female rats and in male mice. Mortality rates of either sex or either species were not affected by treatment.

In rats, benign tumors (adenoma and fibroadenoma) of the mammary gland in females showed a dose-related trend (P = 0.039) compared with matched, but not pooled,

controls (8/72 pooled controls, 0/9 matched controls, 5/50 low-dose, 10/49 high-dose). Adenocarcinoma of the mammary gland occurred in two additional low-dose females. The incidences of these tumors in either of the treated groups were not significantly higher than those in the control groups using either matched or pooled controls. Three papillary and follicular-cell adenomas and one papillary adenocarcinoma of the thyroid occurred in the low-dose females, giving a statistically significant increase over the pooled controls ($P = 0.022$), but these thyroid tumors did not occur in the high-dose animals. The dose-related trend was not statistically significant using either pooled or matched controls, and the incidence in the low-dose group is not greater than that in the historical controls. In male rats, the incidence of hemangiomas showed a statistically significant dose-related trend ($P = 0.021$) using pooled controls, but the direct comparison of the three hemangiomas in the high-dose group with the pooled-control group was not statistically significant. Furthermore, three hemangiomas is a small number, and the tumors occurred in more than one anatomic site (two in the spleen, one in subcutaneous tissue). The occurrence of these tumors cannot clearly be associated with treatment.

In mice, there were no tumors that were statistically significant in treated groups of either sex.

It is concluded that under the conditions of this bioassay, photodieldrin was not carcinogenic for Osborne-Mendel rats or B6C3F$_1$ mice.

Synonym: 1,1,2,3,3a,7a-hexachloro-exo-5,6-epoxydecahydro-2,4,7-metheno-1H-cyclopenta[a]pentalene

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-18 Bioassay of 3,3'-Iminobis-1-propanol Dimethanesulfonate (Ester) Hydrochloride (IPD) for Possible Carcinogenicity (CAS No. 3458-22-8)

3,3'-Iminobis-1-propanol dimethanesulfonate (ester) hydrochloride, hereinafter called IPD, was synthesized from bis(3-hydroxypropyl)amine and methanesulfonic acid anhydride. It was found to have antitumor activity against a number of experimental tumors that were naturally resistant to nitrogen mustard and has been used in Japan for the treatment of myelogenous leukemia. IPD was selected for carcinogen bioassay as one agent in a series of anticancer drugs that are administered chronically in the treatment of human cancer.

A bioassay of IPD for possible carcinogenicity was conducted by administering the test chemical intraperitoneally to Sprague-Dawley rats and B6C3F$_1$ mice.

The IPD was injected three times per week to groups of 35 animals, using doses of 12, 24, or 48 mg/kg for the rats, and 20 or 40 mg/kg for the mice. Rats at 12 mg/kg were treated for 52 weeks. Because of the toxicity of the chemical, administration of IPD for the group receiving 24 mg/kg was discontinued at week 34. Rats receiving 48 mg/kg were treated until all had died at week 23 (males) and week 27 (females). Both groups of mice were treated for 52 weeks. All survivors were killed after post-administration periods that varied among groups.

With rats, untreated and vehicle-control groups, each consisting of 10 males and 10 females, were started with the high- and mid-dose groups and additional untreated and vehicle-control groups of the same size were started with the low-dose groups. With mice, untreated and vehicle-control groups each consisted of 15 males and 15 females.

The toxicity of IPD was associated with lower mean body weights and lower rates of survival of both the rats and mice. The shortened life spans, particularly in the rats, reduced the likelihood of the development of tumors.

In rats, peritonitis and fibrous adhesions, possibly, from direct irritation by the test chemical were observed in most treated rats at necropsy. Sarcoma, fibroma, or fibrosarcoma of the peritoneum occurred in two low-dose male, one mid-dose male, and one mid-dose female rats, but not in any control animals. Because of this low incidence, and because irritation by the test chemical have been involved in the pathogenesis, these tumors may have been due to local effects of the chemical.

In mice, lymphomas were observed at the following incidences (males: controls 0/14, low-dose 0/26, high-dose 3/21; females: controls 1/15, low-dose 2/29, high-dose, 6/27). The Tarone test for life-table analysis of the probability of survival without lymphoma indicated a significant positive dose-related increase of lymphomas with a probability level of 0.011 for male mice and 0.003 for female mice.

Squamous-cell carcinoma was noted in the mice (low-dose males 6/26, high-dose females 2/27). Seven of these tumors were observed in subcutaneous tissue in the inguinal region near the sites of injection. Although not statistically significant, this tumor may be associated with administration of IPD.

Tumors of the peritoneum in rats and tumors in the subcutaneous tissue in mice may have been due to local effects related to administration of the test chemical. The lymphomas in mice, although marginally significant, were too few in number to clearly be related to dosing.

Conclusions from this study are limited by early deaths and toxicity, but the appearance of tumors in the peritoneum near the injection sites in both rats and mice indicate the carcinogenic potential of IPD.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Equivocal
Female Mice:	Equivocal

TR-19 Bioassay of Procarbazine for Possible Carcinogenicity (CAS No. 366-70-1)

Procarbazine is a methylhydrazine derivative which has been shown to have effective antineoplastic activity in advanced Hodgkin's disease and in oat-cell carcinoma of the lung. It has also been shown to have carcinogenic activity in rats and mice.

A bioassay of procarbazine for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 34 or 35 males and 35 or 36 females of both species were administered procarbazine at one of two doses, either 15 or 30 mg/kg for rats, and either 6 or 12 mg/kg for mice. Injections were made three times per week for 26 weeks for the rats and 52 weeks for the mice. Following the periods of injection, the dosed animals were observed for a maximum period of 60 weeks for rats and 33 weeks for mice, depending on survival. Vehicle controls, used for statistical evaluation, consisted of 10 rats and 15 mice of each sex, administered saline solution on the same schedule as the test solution; the same numbers of rats and mice served as untreated controls. Pooled controls consisted of the vehicle controls from this bioassay together with the vehicle controls from two other bioassays similarly performed at the same laboratory. The pooled-control groups consisted of 40 rats of each sex and 45 mice of each sex. Surviving rats were killed at 86 weeks and surviving mice were killed at 85 weeks.

Mean body weights of low- and high-dose rats and of high- dose female mice were lower than those of the vehicle controls. Survival rates of both rats and mice showed significant dose-related trends.

In rats, malignant lymphomas, adenocarcinomas of the mammary gland, and the combination of olfactory neuroblastomas, adenocarcinomas, or carcinomas of the brain, olfactory bulb, or cerebrum were induced in statistically significant numbers.

In mice, malignant lymphomas or leukemias, olfactory neuroblastomas or undifferentiated carcinomas, alveolar/bronchiolar adenomas, and adenocarcinomas of the uterus were induced in statistically significant numbers.

It is concluded that under the conditions of this bioassay, procarbazine was carcinogenic for both Sprague-Dawley rats and $B6C3F_1$ mice, producing several types of tumors in both of these two species.

Synonym: N-iso-propyl-α-(2-methylhydrazino)-p-toluamide hydrochloride

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-20 Bioassay of Dapsone for Possible Carcinogenicity (CAS No. 80-08-0)

Dapsone is the parent chemical of the sulfone drugs, and the major therapeutic agent in this group for the treatment of leprosy. It is also administered to treat dermatitis herpetiformis and malaria, and is used in combination with radiotherapy in the treatment of gynecologic neoplasms. Dapsone is also sold for use as an accelerator in epoxy resins.

A bioassay of dapsone for possible carcinogenicity was conducted by administering the test material in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered dapsone at one of two doses, either 600 or 1,200 ppm for rats and either 500 or 1,000 ppm for mice. The rats and mice were treated for 78 weeks; the rats were then observed for 26-28 weeks, the mice for 28-30 weeks. Matched controls consisted of groups of 15 untreated rats and 14 untreated mice of each sex, pooled controls, used for statistical evaluation, consisted of the matched controls combined with 30 male and 30 female untreated rats and 29 male and 29 female untreated mice from similarly performed bioassays of two other test chemicals. All surviving rats were killed at 104-106 weeks, all surviving mice at 106-108 weeks.

Treated rats and mice had lower mean body weights than the corresponding controls; when treatment was discontinued at week 78, both species showed some increase in body weight. Survival among rats was unaffected by treatment with dapsone; adequate numbers of animals survived for meaningful statistical analyses of the incidences of tumors. Dapsone did not adversely affect the survival of mice, as shown by the test for positive dose-related trend. Suppurative bronchopneumonia was found in some mice in all matched-control and treated groups. Several control males died early in the study, while survival of the other groups of mice was not affected until week 75.

Among rats, mesenchymal tumors of the abdominal organs or peritoneal tissues occurred in 13/35 low-dose males and 22/33 high-dose males. None occurred among control males or among control or treated females. The most commonly occurring tumors were fibroma, fibrosarcoma, or sarcoma, NOS (not otherwise specified), of the spleen and the peritoneum. In male rats, these mesenchymal tumors of the spleen occurred in a statistically significant incidence in both treated groups (low-dose 6/34, $P = 0.006$; high-dose 14/32, $P < 0.001$) when compared with pooled controls. In the peritoneum, the incidences of these mesenchymal tumors were significant in both treated groups (low-dose 5/35, $P = 0.014$; high-dose 6/33, $P = 0.005$) when compared with the pooled controls. No tumors related to treatment were found in female rats.

Among the mice, there were no tumors that could clearly be related to treatment.

It is concluded that under the conditions of this bioassay, dapsone was not carcinogenic for female Fischer 344 rats or $B6C3F_1$ mice of either sex. Dapsone was carcinogenic

(sarcomagenic) for male Fischer 344 rats, causing mesenchymal tumors in the spleen and the peritoneum.

Synonym: 4,4-sulfonyldianiline

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-21 Bioassays of Aldrin and Dieldrin for Possible Carcinogenicity (CAS No. 309-00-2) (CAS No. 60-57-1)

Aldrin and dieldrin are organochlorine insecticides of the cyclodiene group. These chemicals are neurotoxins, and their predominant effect is the stimulation of the nervous system. Both aldrin and dieldrin are lipophilic and accumulate in mammalian tissues. Aldrin undergoes metabolic conversion to the epoxide, dieldrin, and because of this structural relationship, reports of the bioassays of both chemicals have been combined in this single report.

Bioassays of technical-grade aldrin and dieldrin for possible carcinogenicity were conducted by administering the test materials in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Aldrin

Groups of 50 rats of each sex were administered aldrin at one of two doses, either 30 or 60 ppm. Male rats were treated for 74 weeks, followed by 37-38 weeks of observation; female rats were treated for 80 weeks, followed by 32-33 weeks of observation. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 58 untreated males and 60 untreated females from similar bioassays of other chemicals. All surviving rats were killed at 111-113 weeks.

Groups of 50 mice of each sex were administered aldrin at one of two doses for 80 weeks, then observed for 10-13 weeks. Time-weighted average doses were 4 or 8 ppm for males and 3 or 6 ppm for females. Matched controls consisted of groups of 20 untreated male mice and 10 female mice; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 92 untreated male and 79 untreated female mice from similar bioassays of other chemicals. All surviving mice were killed at 90-93 weeks.

Mean body weights attained by the rats and mice fed diets containing aldrin were similar to those of the controls during the first year of the study; however, mean body weights of the treated rats were lower than those of the controls during the second year of the study. Hyperexcitability was observed in all treated groups with increasing frequency and severity during the second year. Aldrin produced no significant effect on the mortality of rats or of male mice, but there was a dose-related trend in the mortality of female mice, primarily due to the early deaths in the high-dose groups.

There was an increased combined incidence of follicular-cell adenoma and carcinoma of the thyroid both in male rats fed aldrin (matched controls 3/7, pooled controls 4/48, low-dose 14/38, high-dose 8/38) and female rats fed aldrin (matched controls 1/9, pooled controls 3/52, low-dose 10/39, high-dose 7/46). These incidences were significant in the low-dose but not in the high-dose groups both of males ($P = 0.001$) and females ($P = 0.009$) when compared with the pooled controls. Comparisons with matched controls, however, were not significant.

Cortical adenoma of the adrenal gland was also observed in aldrin-treated rats in significant proportions ($P = 0.001$) in low-dose (8/45) but not in high-dose (1/48) females when compared with pooled controls (0/55). Because these increased incidences were not consistently significant when compared with matched rather than pooled control groups, it is questionable whether the incidences of any of these adrenal tumors were associated with treatment.

In male mice, there was a significant dose-related increase in the incidence of hepatocellular carcinomas (matched controls 3/20, pooled controls 17/92, low-dose 16/49, high-dose 25/45) when compared with either matched controls ($P = 0.001$), or pooled controls ($P < 0.001$). The incidence in the high-dose group was significant when compared with matched controls ($P = 0.002$) or pooled controls ($P < 0.001$).

Dieldrin

Groups of 50 rats and 50 mice of each sex were administered dieldrin at one of two doses. Low-dose rats and both low- and high-dose mice were treated for 80 weeks, followed by observation periods of 30-31 weeks for rats and 10-13 weeks for mice. Treatment of high-dose rats was terminated after 59 weeks and followed by 51-52 weeks of observation. Time-weighted average doses for rats were 29 or 65 ppm; doses for mice were 2.5 or 5 ppm. Matched controls consisted of groups of 10 untreated rats of each sex and 20 untreated male mice and 10 female mice; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with untreated animals from similar bioassays of other chemicals (58 male and 60 female rats, 92 male and 79 female mice). All surviving rats were killed at 110-111 weeks, and all surviving mice at 90-93 weeks.

Mean body weights attained by the rats and mice fed diets containing dieldrin showed little or no differences compared with those of the controls during the first year of the study; however, mean body weights of the treated rats were lower than those of the controls during the second year of the study. Hyperexcitability was observed in all treated groups with increasing frequency during the second year, especially in high-dose rats.

There was a marked increase in the mortality rate of rats during the first 90 weeks of the study. However,

because of the high rates of mortality in the control groups during the remaining 20 weeks, survival could not be shown to be statistically dose responsive.

In rats, there was a significant (P = 0.007) difference between the combined incidence of adrenal cortical adenoma or carcinoma in the low-dose females (6/45) and that in the pooled controls (0/55). Although this tumor was also found in animals treated with aldrin, it is not clearly associated with treatment, because the incidence in the high-dose (2/40) was not significant, and the incidences were not significant when matched, rather than pooled, controls were used for comparison.

In male mice, there was a significant positive dose-related trend (P = 0.020) in the incidence of hepatocellular carcinomas using the pooled controls (pooled controls 17/92, low-dose 12/50, high-dose 16/45). When high-dose males were compared with the pooled controls, the results were also significant (P = 0.025).

It is concluded that under the conditions of these bioassays, none of the tumors occurring in Osborne-Mendel rats treated with aldrin or dieldrin could clearly be associated treatment.

Aldrin was carcinogenic for the liver of male $B6C3F_1$ mice producing hepatocellular carcinomas. With dieldrin, there was a significant increase in the incidence of hepatocellular carcinomas in the high-dose males which may be associated with treatment.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

For Aldrin:	
Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Positive
Female Mice:	Negative
For Dieldrin:	
Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Negative

Note: Dieldrin was also studied by administration in feed to F344 rats (See TR-022, reported 1978).

TR-22 Bioassay of Dieldrin for Possible Carcinogenicity (CAS No. 60-57-1)

Dieldrin is a chlorinated cyclodiene pesticide. It is also a metabolic conversion product of aldrin, another pesticide, and can be expected to appear in the environment following the use of either chemical. Dieldrin was first introduced in the 1950's for use by cotton growers when the chemical was found to be more effective than aldrin, and later, was used as an insecticide for other crops, for public health pest control, and for mothproofing woolen goods.

A bioassay of purified technical-grade dieldrin for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats.

Groups of 24 rats of each sex were administered dieldrin at one of three doses, either 2, 10, or 50 ppm, for 104-105 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. All surviving rats were killed at 104-105 weeks.

Body weights of the rats were essentially unaffected by the treatment, but typical signs of organochlorine intoxication including hyperexcitability, tremors, and coma were observed in high-dose males beginning in week 76 and in high-dose females beginning in week 80. Survival was not adversely affected, and adequate numbers of rats were available for meaningful statistical analyses of the incidences of tumors.

A variety of neoplasms occurred in control and treated rats; however, the incidences were not related to treatment.

It is concluded that under the conditions of this bioassay, dieldrin was not carcinogenic in Fischer 344 rats.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative

Note: Dieldrin was also studied in feed in Osborne-Mendel rats and $B6C3F_1$ mice (See TR-21, reported 1978).

TR-23 Bioassay of Picloram for Possible Carcinogenicity (CAS No. 1918-02-1)

Picloram is a systemic herbicide registered by EPA for only nonfood use to control broadleaf weeds and woody plants. The chemical can replace the plant growth hormone indoleacetic acid, and inhibit the synthesis of protein in plants.

A bioassay of technical-grade picloram for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered picloram in the diet at one of the following doses for 80 weeks. Time-weighted average doses for the rats were 7,437 or 14,875 ppm; those for the mice were 2,531 or 5,062 ppm. The rats were then observed for 33 weeks, the mice for 10 weeks. Matched controls consisted of groups of 10 untreated rats or 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched control groups combined with 33 untreated male and 30 untreated female rats or mice from similar bioassays of three other test chemicals. All surviving rats were killed at 113 weeks; all surviving mice were killed at 90 weeks. Survival was adequate for meaningful statistical analyses of the incidences of tumors in rats and mice of both sexes.

Mean body weights of the high-dose rats were lower than those of matched controls during the first part of the study; however, beginning at approximately 80 weeks, mean weights of controls were lower than those of treated animals. Body weights of the mice were unaffected by the picloram.

In rats, a relatively high incidence of follicular hyperplasia, C-cell hyperplasia, and C-cell adenoma of the thyroid occurred in both sexes. However, the statistical tests for adenoma did not show sufficient evidence for association of the tumor with picloram administration.

An increased incidence of hepatic neoplastic nodules was observed in treated male and female rats as compared with untreated animals. This lesion is considered to be a benign tumor. In male rats the lesion appeared only in three animals of the low-dose treatment group and was not significant when compared with the controls; however, the test for positive dose-related trend in females was significant (pooled controls 0/39, low-dose 5/50, high-dose 7/49, $P = 0.016$) and the incidence in the high-dose group was significant ($P = 0.014$) when compared with that in the pooled-control group.

There was also one hepatocellular carcinoma in a low-dose male rat and one in a high-dose female rat. In both males and females, there was a possibly treatment-related lesion of the liver diagnosed as foci of cellular alteration. The incidences of this latter lesion were, female rats: matched controls 1/10, low-dose 8/50, high-dose 18/49; male rats: matched controls 0/10, low-dose 12/49, high-dose 5/49. Thus, there is evidence that picloram affected the livers of rats of both sexes, but more particularly those of the females.

No tumors were found in male or female mice or male rats at incidences that could be significantly associated with treatment, and it is concluded that picloram was not carcinogenic for $B6C3F_1$ mice or male Osborne-Mendel rats.

In female rats, however, the incidence of neoplastic nodules of the liver, benign tumors, was associated with treatment with picloram. It is concluded that under the conditions of the bioassay, the findings are suggestive of the ability of the compound to induce benign tumors in the livers of female Osborne-Mendel rats.

Synonym: 4-amino-3,5,6-trichloropicolinic acid

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Equivocal
Male Mice:	Negative
Female Mice:	Negative

TR-24 Bioassay of Malathion for Possible Carcinogenicity (CAS No. 121-75-5)

Malathion is an organophosphorus insecticide and acaricide first synthesized in the United States in 1952. Malathion primarily affects the nervous system by inhibition of cholinesterase activity and subsequent accumulation of acetylcholine. However, it has a low mammalian toxicity. Malathion is approved for a wide variety of uses as an insecticide and acaricide on field crops, fruits, nut trees, vegetables, livestock, agricultural premises, and land. Tolerances for residues of malathion have been established on many of these products.

A bioassay of technical-grade malathion for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered malathion at one of two doses for 80 weeks, then observed for 33 weeks. Time-weighted average doses were 4,700 or 8,150 ppm. Matched controls consisted of groups of 15 untreated rats of each sex; pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female rats from similar bioassays of four other test chemicals. All surviving rats were killed at 108-113 weeks.

Groups of 50 mice of each sex were administered malathion at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 14 or 15 weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched controls combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 94 or 95 weeks.

Mortality in either rats or mice was not significantly related to the administration of malathion. Sufficient numbers of animals were at risk in the dosed and control groups of rats and mice of each sex for development of late-appearing tumors.

In female rats, three follicular-cell carcinomas and one follicular-cell adenoma of the thyroid occurred in the high-dose group, and three follicular-cell hyperplasias occurred in the low-dose group. The incidence of these tumors showed a statistically significant ($P = 0.026$) dose-related trend; however, the results of the Fisher exact test for direct comparison between the dosed and control groups were not significant. More dosed males than dosed females had either tumors or hyperplasia of the follicular cells of the thyroid; however, because of the higher incidence of tumors among the male controls, none of the results of the statistical tests were significant. These thyroid tumors were not considered to be associated with the administration of malathion.

In male mice, hepatocellular carcinoma occurred at the following incidences: matched controls 2/10, pooled controls 5/49, low-dose 7/48, high-dose 11/49. In addition, neoplastic nodules occurred in 3/49 pooled-control and 6/49 high-dose animals. When the combined incidence of these neoplasms in the dosed animals was compared with that of the pooled controls, the dose-related trend was $P = 0.019$ and the direct comparison of the high-dose group with the control group was $P = 0.031$. Thus, none of the direct comparisons of dosed groups with controls were significant using the Bonferroni criteria. In addition, the historical controls from this laboratory had several control groups with incidences of 35-40% hepatocellular carcinoma, rates which are comparable with the incidence of this tumor in the dosed male mice of the present study. Thus, these liver tumors are not considered to be associated with the administration of malathion. The incidences of liver tumors in dosed females were not statistically

significant when compared with that in control animals.

It is concluded that under the conditions of this bioassay, there was no clear evidence of the association of the tumor incidence with the administration of malathion to Osborne-Mendel rats or $B6C3F_1$ mice.

Synonym: S-(1,2-dicarbethoxyethyl)-*O,O*-dimethyldithiophosphate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

Note: Malathion was subsequently studied by administration in feed to F344 rats (See TR-192, reported 1979).

TR-25 Bioassay of Chloramben for Possible Carcinogenicity (CAS No. 133-90-4)

Chloramben has been used since 1958 as a preemergent herbicide to control shallow, germinating, broadleaf weeds and annual grasses. Applied as a spray at the time of planting, chloramben remains effective in the soil for several weeks until crops have become well established.

A bioassay of technical-grade chloramben for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and $B6C3F_1$ mice. Groups of 50 rats and 50 mice of both sexes were administered chloramben at one of two doses, either 10,000 or 20,000 ppm. The rats were treated for 80 weeks, then observed for 32 or 33 weeks; the mice were treated for 80 weeks, then observed for 11 or 12 weeks. Matched controls consisted of groups of 10 untreated rats and 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of these matched controls combined with 75 untreated male and 75 untreated female rats or 70 untreated male and 70 untreated female mice from similarly performed bioassays of six other test chemicals. Surviving rats were killed at 112 or 113 weeks; surviving mice were killed at 91 or 92 weeks.

Body weights and mortality of the treated animals were not markedly affected by chloramben under the conditions of the bioassay. The various clinical signs observed were common to both treated and control groups.

In male rats, hemangiomas occurred at a significantly higher incidence in the low-dose animals than in the pooled controls (controls 0/73, low-dose 5/48, $P = 0.009$). This lesion was not considered to be related to the administration of chloramben, since the tumor did not occur at a significantly higher incidence in the high-dose group than in the pooled-control group, and the incidences did not show a significant dose-related trend.

In both male and female mice, the incidences of hepatocellular carcinoma showed significant dose-related trends using pooled controls (for females: controls 9/69, low dose 16/48, high-dose 14/48, $P = 0.029$; for females controls 2/67, low-dose 7/48, high-dose 10/50, $P = 0.004$). Direct comparisons showed significantly higher incidences of the tumor in the low-dose males ($P = 0.008$) and in the high-dose females ($P = 0.003$) than in the pooled controls. Probability levels of $P = 0.028$ in high-dose males and $P = 0.027$ in low-dose females were attained. In male mice, however, the incidence of hepatocellular carcinoma was considered to be only marginally associated with the administration of chloramben because of the variations in the spontaneous incidence of this lesion in male mice encountered at this laboratory.

In conclusion, under the conditions of this bioassay, there were no tumors in Osborne-Mendel rats that were significantly related to administration of the chemical. In $B6C3F_1$ female mice, chloramben was carcinogenic, producing hepatocellular carcinomas in treated animals.

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Positive

TR-26 Bioassay of Nitrofen for Possible Carcinogenicity (CAS No. 1836-75-5)

Nitrofen, a substituted diphenyl ether, is one of several agricultural pesticides selected for bioassay by the National Cancer Institute because of a lack of adequate chronic toxicity data.

A bioassay of technical-grade nitrofen for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Nitrofen was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The time-weighted average high and low dietary concentrations of nitrofen were 3,656 and 2,300 ppm for male rats, 2,600 and 1,300 ppm for female rats, and 4,696 and 2,348 ppm for both male and female mice, respectively. After a 78-week treatment period, observation of the low dose and control male and all female rats continued for an additional 32 weeks; observation of the high dose male rats continued for an additional 4 weeks. All mice were observed for an additional 12 weeks after the 78-week treatment period.

For each species, 20 animals of each sex were placed on test as controls. No nitrofen was added to their diet.

The incidence of carcinomas of the pancreas had a statistically significant positive association with concentration of nitrofen in the diet of female rats. The incidence of this tumor in high dose female rats was significant when compared to controls. Poor survival related to chemical toxicity precluded the evaluation of the carcinogenicity of nitrofen in male rats.

In mice of both sexes, the incidence of hepatocellular carcinoma at both high and low dose levels was highly significant when compared to the controls. The incidence of hemangiosarcoma of the liver had a statistically signifi-

cant relationship with nitrofen concentration in the diet for mice of both sexes, and the incidence in high dose male mice was significant when compared to controls.

The results of this study indicate that orally administered technical-grade nitrofen is a liver carcinogen in B6C3F$_1$ mice of both sexes. Nitrofen is also carcinogenic to female Osborne-Mendel rats.

Synonyms: 2,4-dichloro-1-(4-nitrophenoxy)-benzene; 2,4-dichlorophenyl-p-nitrophenyl ether; nitrophene, Tok E-25; Nip

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

Note: Nitrofen was subsequently studied by administration in feed to F344 rats and B6C3F$_1$ mice (See TR-184, reported 1979).

TR-27 Bioassay of 1,1,2,2-Tetrachloroethane for Possible Carcinogenicity (CAS No. 79-34-5)

1,1,2,2,-Tetrachloroethane, an aliphatic chlorinated hydrocarbon, is one of a group of halogenated solvents selected for bioassay by the National Cancer Institute. Solvents were selected on the basis of large-scale production, extensive use, and lack of adequate chronic toxicity data.

A bioassay for possible carcinogenicity of technical-grade 1,1,2,2-tetrachloroethane was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. At initiation of the study the rats were approximately 7 weeks old, and the mice were approximately 5 weeks old. 1,1,2,2-Tetrachloroethane in corn oil was administered by gavage, at either of two dosages, to two groups of 50 male and 50 female animals of each species, 5 days a week. Treatment was over a period of 78 weeks, followed by observation periods of 32 weeks for the rats and 12 weeks for the mice. The high and low time-weighted average dosages were, respectively, 108 and 62 mg/kg/day for male rats, 76 and 43 mg/kg/day for female rats, and 282 and 142 mg/kg/day for the mice of both sexes.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were intubated with corn oil at the same rate as the high dose animals. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

Among mice, hepatocellular carcinomas were observed in 2/16 (13 percent) male untreated controls, 1/18 (6 percent) male vehicle controls, 13/50 (26 percent) low dose males, 44/49 (90 percent) high dose males, 0/18 female untreated controls, 0/20 female vehicle controls, 30/48 (63 percent) low dose females, and 43/47 (91 percent) of the high dose females. This incidence of hepatocellular carcinoma indicated a highly significant ($P<0.001$) positive dose-related trend in mice of both sexes.

No statistically significant incidence of neoplastic lesions was observed in male or female rats. However, two hepatocellular carcinomas and one neoplastic nodule, which are rare tumors in the male Osborne-Mendel rat, were observed in the high dose males.

Under the conditions of this study, orally administered 1,1,2,2-tetrachloroethane is a liver carcinogen in B6C3F$_1$ mice of both sexes. The results do not provide conclusive evidence for the carcinogenicity of 1,1,2,2-tetrachloroethane in Osborne-Mendel rats.

Synonyms: acetylene tetrachloride; sym-tetrachloroethane

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-28 Bioassay of Dibromochloropropane for Possible Carcinogenicity (CAS No. 1836-75-5)*

Dibromochloropropane, a halogenated aliphatic hydrocarbon and agricultural pesticide, was one of several widely used pesticides selected for bioassay by the National Cancer Institute. At this time there was a lack of adequate chronic toxicity data on this compound.

A bioassay for possible carcinogenicity of technical-grade dibromochloropropane (DBCP) was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. DBCP in corn oil was administered by gavage 5 days a week, at either of two dosages, to groups of 50 male and 50 female animals of each species.

Initial dosage levels for the chronic bioassay were selected on the basis of a preliminary subchronic toxicity test. Subsequent dosage adjustments were made during the course of the chronic bioassay. The time-weighted average dosages of DBCP in the chronic study were 29 mg/kg/day for the high dose rats of both sexes, and 15 mg/kg/day for the low dose rats of both sexes. The time-weighted average concentrations for the high dose male and female mice were 219 and 209 mg/kg/day, respectively. The time-weighted average concentrations for the low dose male and female mice were 114 and 110 mg/kg day, respectively.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were intubated with corn oil at the same time that dosed animals were intubated with DBCP mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals received no gavage treatments.

DBCP was administered to the high dose male and high dose female rats for 64 weeks prior to sacrifice, and to the low dose female rats for 73 weeks prior to sacrifice. The low dose male rats were treated for 78 weeks followed by an additional 5 weeks of observation. The high dose male and female mice were treated for 47 weeks prior to sacrifice; the low dose male mice were treated for 59 or 60 weeks prior to sacrifice, and the low dose female mice were treated for 60 weeks prior to sacrifice.

In rats and mice of both sexes, statistically significant incidences of squamous-cell carcinomas of the forestomach occurred in each dosed group and a significant positive association existed between dosage level and tumor incidence. The incidences of adenocarcinomas of the mammary gland were statistically significant in female rats when the treated groups were compared to the controls. Toxic nephropathy was also observed at elevated incidences in all of the treated rats and mice when compared to their respective untreated or vehicle control groups.

Under the conditions of this study, DBCP is a stomach carcinogen in rats and mice of both sexes and is carcinogenic to the mammary gland in female rats.

Synonyms: 1,2-dibromo-3-chloropropane; Nemagon®; Fumazone®; DBCP

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

*The technical report states that the Chemical Abstract Service (CAS) Ninth Collective Index (1977) name for this compound is 1,2-dibromo-3-chloropropane (CAS No. 96-12-8). This is the CAS number used to track this study in the NTP CHEMTRACK database.

Note: Dibromochloropropane was subsequently studied by inhalation to F344 rats and B6C3F$_1$ mice (See TR-206, reported 1982).

TR-29 Bioassay of 2-Methyl-1-Nitroanthraquinone for Possible Carcinogenicity (CAS No. 129-15-7)

2-Methyl-1-nitroanthraquinone, an intermediate in the synthesis of anthraquinone dyes, was selected for bioassay by the National Cancer Institute in an attempt to elucidate those chemicals which may be responsible for the increased incidence of bladder cancer among workers in the dye manufacturing industry. Aromatic nitro compounds are one of several classes of chemicals thought to contribute to the increased cancer risk in this industry.

A bioassay of 2-methyl-1-nitroanthraquinone for possible carcinogenicity was conducted using Fischer 344 rats. 2-Methyl-1-nitroanthraquinone was administered in the feed at either of two concentrations to groups of 50 male and 50 female animals. The high and low dietary concentrations used were 0.12 and 0.06 percent, respectively, for the male and female rats. After a 78-week treatment period, observation of the rats continued for an additional 31 weeks. Fifty rats of each sex were placed on test as controls. No 2-methyl-1-nitroanthraquinone was added to their diet.

Survival in both the male and female rats was adequate for a meaningful statistical analysis of late-developing tumors; however, there was a significant positive association between increased dosage and elevated mortality in female rats.

Hepatocellular carcinomas and neoplastic nodules of the liver occurred in both the male and female treated rats. A statistically significant association between increased dosage and an elevated incidence of hepatocellular carcinomas was indicated by the Cochran-Armitage test for the males (1/48, 5/48, and 9/49 in control, low dose, and high dose, respectively); however, the Fisher exact tests supported these results only for the high dose males. The incidence of neoplastic nodules was statistically significant in the male rats (0/48, 2/48, and 6/49 in control, low dose, and high dose, respectively), as indicated by the Cochran-Armitage test and supported by the Fisher exact test for the high dose group. When those rats having either hepatocellular carcinomas or neoplastic nodules of the liver were combined and evaluated simultaneously, the Cochran-Armitage tests indicated statistically significant associations between increased dosages and elevated tumor incidences in both the males and females. This was supported by the Fisher exact tests for males but not for females. The incidences of one tumor type, subcutaneous fibroma, were found to be statistically significant in both male and female rats. No other tumors occurred in treated animals in statistically significant incidences when compared to controls.

Squamous-cell papillomas and squamous cell carcinomas of the forestomach were observed only in high dose rats. Although the incidences of these gastric tumors were not statistically significant, historical data indicate that these tumors are rare in Fischer 344 rats. The occurrence of these tumors in high dose rats, together with the frequent occurrence of nonneoplastic proliferative lesions of the forestomach in treated rats, indicates that the occurrence of these tumors was related to administration of 2-methyl-1-nitroanthraquinone. An increased incidence of bladder tumors (papillomas, transitional-cell papillomas, and sarcomas) was observed among female rats.

Under the conditions of this bioassay, the results indicate that orally administered 2-methyl-1-nitroanthraquinone is carcinogenic in male Fischer 344 rats, producing hepatocellular carcinomas. Increased incidences of subcutaneous fibromas in both male and female Fischer 344 rats were also associated with the administration of the compound. Tumors of the forestomach and bladder in these animals may also have been related to the administration of the test chemical.

Synonyms: 2-methyl-1-nitro-9,10-antracenedione; 1-nitro-2-methyl-anthraquinone

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-30 Bioassay of Diarylanilide Yellow for Possible Carcinogenicity (CAS No. 6358-85-6)

Diarylanilide yellow, one member of a family of organic azo pigments known as benzidine yellows, was selected for bioassay by the National Cancer Institute in an attempt to elucidate those dyes and dye intermediates which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry.

A bioassay of technical-grade diarylanilide yellow for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. Diarylanilide yellow was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low dietary concentrations used in the chronic study for the male and female rats and mice were 5.0 and 2.5 percent, respectively, of the chemical in the feed. After a 78-week treatment period, observation of the rats continued for an additional 28 weeks and observation of the mice continued for an additional 19 weeks for high dose males and low and high dose females and 18 weeks for low dose males. For each species, 50 animals of each sex were placed on test as controls, and fed only the basal diet.

The high concentration administered to both species in this study was the maximum recommended in the Guidelines for Carcinogen Bioassay in Small Rodents. These guidelines indicate that a chronic dietary level of 5 percent, or 50,000 ppm, should not be exceeded even when no signs of toxicity are observed during subchronic testing, except under special circumstances (e.g., when the compound is a major component of the human diet). No toxic effects were reported during subchronic testing and diarylanilide yellow did not qualify for exception; therefore, the highest permissible concentration (5 percent) was utilized in the chronic bioassay.

The dietary concentrations of diarylanilide yellow administered during the chronic bioassay had no significant effect on survival or body weight gain in either species. Except for yellow staining and some isolated neoplasms, the only adverse clinical sign or pathologic lesion observed in treated rats or mice was basophilic cytoplasm changes in hepatocytes of treated rats.

In both species the survival in all groups was adequate for statistical analysis of late-appearing tumors.

No treatment-related increase in the incidence of neoplasms or nonneoplastic lesions was evident in treated rats or mice. A few unusual findings were observed in both species, including single cases of metastatic chordoma and osteogenic sarcoma in rats, and single cases of squamous-cell carcinoma of the ear, infiltrating duct carcinoma of the mammary gland, and subcutaneous mastocytoma in mice.

The results of the study did not provide evidence for the carcinogenicity of diarylanilide yellow in Fischer 344 rats or $B6C3F_1$ mice.

Synonyms: 2,2'-[(3,3'-dichloro(1,1'-biphenyl)-4,4'-diyl)-bis (azo)] bis(3-oxo-N-phenyl)-butanamide; C.I. Pigment Yellow 12; Diarylide yellow

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-31 Bioassay of Tolbutamide for Possible Carcinogenicity (CAS No. 64-77-7)

Tolbutamide was the first oral hypoglycemic agent used in the management of diabetes. It is one of the arylsulfonylurea hypoglycemics, a group which included tolazamide, chlorpropamide, and acetohexamide. All of these compounds function by stimulating the secretion of insulin by the pancreas and, therefore, are used only in patients with at least minimal pancreatic function, as in maturity-onset diabetics.

A bioassay of tolbutamide for possible carcinogenicity was conducted by administering the test material in the diet to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered tolbutamide at one of two doses, either 12,000 or 24,000 ppm, 5 days a week for 78 weeks, then observed for an additional 28 weeks. Matched-control groups consisted of 15 untreated rats of each sex. All surviving rats were killed at 106 or 107 weeks.

Groups of 35 mice of each sex were administered tolbutamide at one of two doses, either 25,000 or 50,000 ppm, 5 days a week for 78 weeks, then observed for an additional 24-26 weeks. Matched-control groups consisted of 15 untreated mice of each sex. All surviving mice were killed at 102-104 weeks.Mean body weights of the treated rats and mice were lower than those of the corresponding matched controls during the entire study; however, survival was not significantly affected by treatment in either species. In both sexes of both species, survival was considered to be adequate for meaningful statistical analyses of the incidence of tumors.

In both the rats and the mice, a variety of neoplasms were found in both tolbutamide-treated and control groups. None of the neoplasms were present at a statistically significant increased incidence in treated groups of either species as compared with control groups and were not considered to be compound related.

It is concluded that under the conditions of this bioassay, tolbutamide was not carcinogenic for either Fischer 344 rats or $B6C3F_1$ mice.

Synonym: 1-butyl-3-(p-methylbezenesulfonyl)urea

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-32 Bioassay of Isophosphamide for Possible Carcinogenicity (CAS No. 3778-73-2)

A bioassay of the anticancer drug isophosphamide for possible carcinogenicity was conducted by injecting the test chemical intraperitoneally into Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were given the injections at one of two doses three times per week for 52 weeks. Doses for rats were either 6 or 12 mg/kg, and for mice either 10 or 20 mg/kg. After the period of administration of the isophosphamide, the surviving rats were observed for 31 weeks and the mice for 28 weeks. Untreated controls as well as vehicle controls (buffered saline) were used. The matched vehicle-control groups each consisted of 10 rats or 15 mice of each sex; pooled vehicle-control groups, used for statistical evaluation, consisted of the matched vehicle controls of each species combined with 20 male and 20 female matched vehicle-control rats or 15 male and 15 female matched vehicle-control mice from a similar bioassay of another test chemical. All surviving rats were killed at 79-84 weeks, all surviving mice at 79-81 weeks.

Mean body weights of the high-dose rats of either sex were lower than those of the matched vehicle controls after approximately 25 weeks on study. Survival was low among the high-dose male and female rats, but in the low-dose groups it was adequate for meaningful statistical analyses of the incidences of tumors. Mean body weights of the mice did not show any consistent effect from the isophosphamide treatment. Survival was adequate for meaningful statistical analyses in both groups of female mice, while survival of the males was 31% for both treated groups at the end of the study.

In male rats, tumors of the hematopoietic system included six malignant lymphomas and two granulocytic leukemias. With the unadjusted analysis, these tumors showed a dose-related trend in male rats using pooled vehicle controls (controls 0/29, low-dose 3/32, high-dose 5/34, $P = 0.032$) and a higher incidence in the high-dose males than in the pooled vehicle controls ($P = 0.040$). These tumors were not significant when compared with matched vehicle controls using adjusted analyses, and they cannot clearly be associated with treatment. However, it should be noted that five rats with these tumors were observed in the high-dose group whose median survival was only 35 weeks.

In female rats, the incidence of uterine leiomyosarcoma was significant in the low-dose group using pooled vehicle controls (controls 0/27, low-dose 15/32, $P<0.001$). There was also a significant incidence of mammary fibroadenoma among low-dose females using pooled vehicle controls (controls 8/28, low-dose 28/33, $P<0.001$). The incidence of each tumor was also significant when compared with matched vehicle controls using time-adjusted analyses. The low survival of the high-dose group (median time on study, 35 weeks) may explain the lower incidences of the uterine leiomyosarcoma and the mammary fibroadenoma in this group. In some rats, the leiomyosarcomas metastasized to the lungs, urinary bladder, spleen, and other abdominal sites.

In female mice, the incidence of malignant lymphoma of the hematopoietic system showed a significant dose-related trend using either matched vehicle controls (controls 0/14, low-dose 3/32, high-dose 13/34, $P=0.001$) or pooled vehicle controls (controls 1/29, $P<0.001$). Further, incidences of this tumor in the high-dose females were significantly higher than incidences in the matched vehicle ($P=0.005$) or pooled vehicle ($P=0.001$) controls.

It is concluded that under the conditions of this bioassay, isophosphamide was not carcinogenic in male Sprague-Dawley rats or in male $B6C3F_1$ mice. However, the incidence of leiomyosarcomas of the uterus indicates that isophosphamide was carcinogenic in female Sprague-Dawley rats, and the incidence of fibroadenoma of the mammary gland in female rats was associated with treatment with isophosphamide. Isophosphamide was carcinogenic in female $B6C3F_1$ mice, producing malignant lymphomas of the hematopoietic system.

Synonyms: 3-(2-chloroethyl)-2-[2-chloroethyl)amino]-tetrahydro-1,3,2-oxazaphosphorine-2-oxide; ifosfamide

Report Date: 1977

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Positive

TR-33 Bioassay of Tetrachlorvinphos for Possible Carcinogenicity (CAS No. 961-11-5)

Tetrachlorvinphos is an organophosphorous pesticide introduced in 1966 by Shell Development Company. It is registered for use against various pests of fruits, vegetables, ornamental plants, forest trees, and livestock, and for use on agricultural premises, agricultural equipment, and recreational areas.

A bioassay of technical-grade tetrachlorvinphos for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered tetrachlorvinphos at one of two doses for 80 weeks, then observed for 31 additional weeks. Time-weighted average doses were either 4,250 or 8,500 ppm. Matched controls

consisted of groups of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 45 untreated male and 45 untreated female rats from similar bioassays of four other test chemicals. All surviving rats were killed at 111 weeks.

Groups of 50 mice of each sex were administered tetrachlorvinphos at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 12 additional weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 90-92 weeks.

The mean body weights of the treated rats and mice were generally lower than those of the matched controls; however, the mortality rate was affected adversely by tetrachlorvinphos only in the male rats. Survival of all groups of rats and mice was adequate for meaningful statistical analyses of the incidence of tumors, except for a matched-control group of female rats for which the survival was abnormally low.

In rats, C-cell adenoma of the thyroid showed a significant dose-related trend in the females, using pooled controls (controls 1/46, low-dose 2/50, high-dose 7/46, $P = 0.013$), and by direct comparison, an increased incidence in the high-dose group ($P = 0.027$). High incidences of C-cell hyperplasia in treated males and females further indicated a chemical-related effect on proliferative lesions of the thyroid. Cortical adenoma of the adrenal also showed a significant dose-related trend in the females, using pooled controls (controls 0/50, low-dose 2/49, high-dose 5/50, $P = 0.017$), and by direct comparison, an increased incidence in the high-dose group ($P = 0.022$). Hemangioma of the spleen occurred in male rats at a significantly higher incidence in the low-dose group than in the pooled controls (controls 0/52, low-dose 4/48, $P = 0.049$); however, neither the incidence in the high-dose group (0/47) nor the test result for dose-related trend was statistically significant.

In mice, hepatocellular carcinoma in males showed a highly significant dose-related trend, using either matched controls (controls 0/9, low-dose 36/50, high-dose 40/50, $P < 0.001$) or pooled controls (controls 5/49, $P < 0.001$). This finding was supported by direct comparisons of low- and high-dose groups of males with matched- or pooled-control groups, which showed highly significant increases in incidences of the tumor in the treated groups in all instances ($P < 0.001$). In females, the incidence of hepatocellular carcinoma was not significant; however, the incidence of neoplastic nodule was significantly higher in both the low- and high-dose groups than in the pooled controls (controls 1/48, low-dose 14/49, $P < 0.001$; high-dose 9/47, $P = 0.007$), using pooled controls for tests for both doses. Because of this higher incidence in the low-dose group than in the high-dose group, there was a significant departure from linear trend ($P = 0.006$).

Granulomatous lesions of the liver were found in high proportions in both treated rats and treated mice, but none were found in matched controls.

It is concluded that under the conditions of this bioassay, the administration of technical-grade tetrachlorvinphos in Osborne-Mendel rats was associated with proliferative lesions of the C cells of the thyroid and cortical adenomas of the adrenal in females. In female $B6C3F_1$ mice, the incidence of neoplastic nodule of the liver was associated with treatment, and in male mice tetrachlorvinphos was carcinogenic, causing hepatocellular carcinoma of the liver.

Synonym: 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-34 Bioassay of Trifluralin for Possible Carcinogenicity (CAS No. 1582-09-8)

Trifluralin, a tertiary aromatic amine and dinitrotoluene derivative, is one of several widely used agricultural pesticides selected for bioassay by the National Cancer Institute because of a lack of adequate chronic toxicity data.

A bioassay for possible carcinogenicity of technical-grade trifluralin was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Analysis of the technical product established the presence of 84 to 88 ppm dipropylnitrosoamine. The product was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Fifty animals of each sex were placed on test as controls for the rat bioassay, while 20 of each sex were utilized as controls for the mouse study. The time-weighted average high and low dietary concentrations of trifluralin were, respectively, 8,000 and 4,125 ppm for male rats, 7,917 and 4,125 ppm for female rats, 3,744 and 2,000 ppm for male mice, and 5,192 and 2,740 ppm for female mice. After a 78-week treatment period, there was an additional observation period of 33 weeks for rats and 12 weeks for mice.

For female mice the association between increased dosage and elevated incidence of hepatocellular carcinomas was significant (0/20, 12/47, and 21/44 of the control, low dose, and high dose, respectively) as was the relationship between dose and incidence of alveolar/bronchiolar adenomas. Significance of incidence for both types of tumors was supported by tests for significance at each dose level. Squamous-cell carcinomas of the stomach were observed in dosed female mice, but not in controls. Although incidences of these tumors were not statistically significant, they are unusual lesions in $B6C3F_1$ mice and are considered to be treatment-related.

Neoplasms observed in treated rats were types that have occurred spontaneously in this strain and were apparently unrelated to trifluralin treatment.

Evaluation of the results of this bioassay indicates that

technical-grade trifluralin is a carcinogen in female B6C3F$_1$ mice, being associated in these animals with an elevated incidence of hepatocellular carcinomas, alveolar/bronchiolar adenomas and squamous-cell carcinomas of the forestomach. Sufficient evidence was not provided for the carcinogenicity or tumorigenicity of trifluralin in male B6C3F$_1$ mice or in Osborne-Mendel rats of either sex.

Synonyms: 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl) benzeneamine; α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine; N,N,-dipropyl-4-trifluoromethyl-2,6-dinitroaniline; Treflan

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Positive

TR-35 Bioassay of Methoxychlor for Possible Carcinogenicity (CAS No. 72-43-5)

Methoxychlor, a synthetic organochlorine insecticide and structural analog of DDT, was one of several widely used agricultural pesticides selected for bioassay by the National Cancer Institute because of a lack of adequate chronic toxicity data. The suspect state of all DDT-related chemicals was an important additional factor in its selection.

A bioassay for possible carcinogenicity of technical-grade methoxychlor was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. Methoxychlor was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. For each species, 20 animals of each sex were placed on test as controls. The time-weighted average high and low dietary concentrations of methoxychlor were, respectively, 845 and 448 ppm for male rats, 1,385 and 750 ppm for female rats, 3,491 and 1,746 ppm for male mice, and 1,994 and 997 ppm for female mice. After a treatment period of 78 weeks, the rat groups were observed for an additional 34 weeks and the mouse groups for an additional 15 weeks. A dose-related mean group body weight depression was observed in both rats and mice, but no effect on survival was detected.

Under the conditions of this study, methoxychlor was not found to be carcinogenic in Osborne-Mendel rats or B6C3F$_1$ mice of either sex.

Synonyms: 1,1'-(2,2,2-trichloroethylidene)bis (4-methoxy)-benzene; 1,1,1-trichloro-2,2'-bis(p-methoxyphenyl)-ethane; dianisyl trichloroethane; Dimethoxy DDT; DMDT

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-36 Bioassay of Anthranilic Acid for Possible Carcinogenicity (CAS No. 118-92-3)

Anthranilic acid is an aromatic amine which occurs physiologically as a metabolite of the amino acid tryptophan. It is used commercially as an intermediate in dye synthesis.

A bioassay of anthranilic acid for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered anthranilic acid at one of the following doses, either 15,000 or 30,000 ppm for the rats, and either 25,000 or 50,000 ppm for the mice, 5 days per week for 78 weeks, then observed for an additional 26-27 weeks. Matched controls consisted of groups of 15 rats and 15 mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 15 untreated male and 15 untreated female animals of each species from a similar bioassay of another test chemical Except for the matched-control male mice, all surviving animals in the study were killed at 104-106 weeks. Half of the matched-control male mice, which were accidentally killed at week 12, were excluded from the report; the remaining matched-control males died by week 94.

Mean body weights of the low- and high-dose male and high-dose female rats were lower than those of the corresponding matched controls for the duration of the study. The weights of the low-dose females were similar to those of the matched controls for the first 45 weeks, after which they declined slightly. The weights of the low-dose male mice were similar to those of the matched controls, while those of the high-dose males and of the low- and high- dose females were slightly lower.

Survival of both treated and matched-control groups of rats of both sexes was high; survival of treated mice of both sexes and of female matched controls, although lower than that of the rats, was sufficient for meaningful statistical analyses of the incidences of tumors.

In rats, a variety of neoplasms were observed in both treated and control animals. Few malignant tumors were found, and no tumors occurred in treated animals in statistically significant incidences when compared with control animals.

In mice, a variety of neoplasms were observed in both treated and control animals. These neoplasms are not uncommon in this strain of mouse, and none occurred in treated animals in statistically significant incidences when compared with control animals.

It is concluded that under the conditions of this bioassay, anthranilic acid was not carcinogenic for either Fischer 344 rats or B6C3F$_1$ mice.

Synonym: 2-aminobenzoic acid

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-37 Bioassay of Toxaphene for Possible Carcinogenicity (CAS No. 8001-35-2)

Toxaphene is an organochlorine insecticide that belongs to the class of compounds known as polychlorinated bicyclic terpenes with chlorinated camphenes predominating; an insecticide marketed as Strobane-T® (Tenneco Chemical Co., Piscataway, N.J.) is identical with toxaphene.

A bioassay of technical-grade toxaphene for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered toxaphene at one of two doses for 80 weeks, then observed for 28 or 30 weeks. Time-weighted average doses for males were 556 or 1,112 ppm; for females they were 540 or 1,080 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups for toxaphene combined with 45 untreated male and 45 untreated female rats from similar bioassays of five other test chemicals. All surviving rats were killed at 108-110 weeks.

Groups of 50 mice of each sex were administered toxaphene at one of two doses for 80 weeks, then observed for 10 or 11 weeks. Time-weighted average doses were 99 or 198 ppm for both males and females. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups for toxaphene combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 90-91 weeks.

Mean body weights attained by low- and high-dose female rats and high-dose male mice were lower than those of matched controls, but weights of other dosed groups were essentially unaffected by the toxaphene. Other clinical signs of toxicity in rats included generalized body tremors at week 53 in high-dose male and female animals, and later, leg paralysis, ataxia, epistaxis, hematuria, and vaginal bleeding, predominantly in the dosed groups of rats of each sex. Abdominal distention, diarrhea, dyspnea, and rough hair coats were common to both dosed rats and dosed mice. There were dose-related decreases in survival rates in mice but not in rats. Sufficient numbers of both rats and mice were at risk for the development of late-appearing tumors.

In the male rats, the incidence of follicular-cell carcinomas or adenomas of the thyroid was dose related ($P = 0.007$) using the pooled controls (matched controls 1/7, pooled controls 2/44, low-dose 7/41, high-dose 9/35). In the females, the incidence of follicular-cell adenomas of the thyroid was dose related using either the matched ($P = 0.022$) or pooled ($P = 0.008$) controls (matched controls 0/6, pooled controls 1/46, low-dose 1/43, high-dose 7/42). Direct comparisons of dosed and pooled-control groups but not matched controls showed significantly increased incidences of follicular-cell carcinomas or adenomas in the high-dose males ($P = 0.008$) and of follicular-cell adenomas in the high-dose females ($P = 0.021$). Two follicular-cell tumors in the high-dose males were carcinomas; all other follicular-cell tumors in the rats were adenomas.

In the mice, the incidence of hepatocellular carcinomas was dose related ($P < 0.001$) for both males (matched controls 0/10, pooled controls 4/48, low-dose 34/49, high-dose 45/46) and females (matched controls 0/9, pooled controls 0/48, low-dose 5/49, high-dose 34/49), using either matched or pooled controls. Direct comparisons showed that the incidences of hepatocellular carcinomas in low- and high-dose male mice and high-dose female mice were all significantly higher ($P < 0.001$) than those in the respective matched or pooled controls. Statistical significance was maintained when the incidence of hepatocellular carcinomas was combined with that of neoplastic nodules of the liver.

It is concluded that under the conditions of this bioassay, toxaphene was carcinogenic in male and female $B6C3F_1$ mice, causing increased incidences of hepatocellular carcinomas. The test results also suggest carcinogenicity of toxaphene for the thyroid of male and female Osborne-Mendel rats.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Positive
Female Mice:	Positive

TR-38 Bioassay of Aroclor® for Possible Carcinogenicity (CAS No. 27323-18-8)*

Aroclor® is the registered trademark of the Monsanto Chemical Company for their polychlorinated biphenyls (PCBs). PCBs were developed in 1929 primarily for use as heat transfer fluids and dielectrics (insulators). Aroclor® 1254, a biphenyl containing approximately 54% chlorine, is a nonflammable heat transfer agent which functions in the range of 250-360° C.

A bioassay of Aroclor® 1254 for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats.

Groups of 24 rats of each sex were administered Aroclor® 1254 at one of three doses, either 25, 50, or 100 ppm, for 104-105 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. All surviving rats were killed at 104-105 weeks.

Mean body weights of males and females receiving mid and high doses and females receiving low doses of the chemical were consistently below those of the correspond-

ing controls, beginning at about week 10 of the study. The decrease in survival among males, but not among females, showed a significant dose-related trend. Adequate numbers of animals of both sexes survived for meaningful statistical analyses of the incidences of tumors.

The combined incidences of lymphomas and leukemias showed a significant dose-related trend in males (controls 3/24, low-dose 2/24, mid-dose 5/24, high-dose 9/24, P = 0.009). However, the direct comparisons of each dosed group with those of the matched controls were not statistically significant, and the tumors cannot clearly be related to administration of with Aroclor® 1254.

Hepatocellular adenomas and carcinomas were found in the dosed groups, but not in the controls (males: mid-dose 1/24, high-dose 3/24; females: mid-dose 1/24, high-dose 2/24). Additionally, a high incidence of nonneoplastic hyperplastic nodules was noted in the dosed animals (males: controls 0/24, low-dose 5/24, mid-dose 8/24, high-dose 12/24; females: controls 0/23, low-dose 6/24, mid-dose 9/22, high-dose 17/24). Although the incidences of tumors were not significant, the occurrence of the hyperplastic nodules appeared to be related to administration of the chemical.

In the stomach, jejunum, or cecum, adenocarcinomas were observed in two dosed males and in two dosed females as well as a carcinoma in one dosed male. None of these lesions was found in control animals in this study. Historical incidences of these tumors at this laboratory (6/600 males [1%], 2/600 females [0.3%] suggest that the lesions, although not statistically significant, may be related to the administration of Aroclor® 1254.

It is concluded that under the conditions of this bioassay, Aroclor® 1254 was not carcinogenic in Fischer 344 rats; however, a high incidence of hepatocellular proliferative lesions in both male and female rats was related to administration of the chemical. In addition, the carcinomas of the gastrointestinal tract may be associated with administration of Aroclor® 1254 in both males and females.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal

*The technical report states that Aroclor® (CAS No. 11097-69-1) was the actual chemical. The CAS number listed in the technical report is for Chloro-1,1'-Biphenyl, the generic form of the compound tested; therefore, the CAS number for Aroclor® is used to track this study in the NTP CHEMTRACK database.

TR-39 Bioassay of Lasiocarpine for Possible Carcinogenicity (CAS No. 303-34-4)

Lasiocarpine is a pyrrolizidine alkaloid that is found in the seeds of *Heliotropium lasiocarpum*, *Heliotropium europaeum*, and several other plant species, all members of the family *Boraginaceae*.

A bioassay of lasiocarpine for possible carcinogenicity was conducted by administering the test chemical in the diet to Fischer 344 rats.

Groups of 24 rats of each sex were administered lasiocarpine at one of three doses, either 7, 15, or 30 ppm, for 104 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. All surviving rats were killed at 104 weeks.

Mean body weights of the high-dose male and female rats were lower than those of the matched-control groups throughout most of the study, while weights of the mid-dose rats were lower only during the second year, and weights of the low-dose groups were unaffected. There was a positive dose-related trend in mortality for both sexes, with none of the high-dose animals, only five of the mid-dose animals, 23 of the low-dose animals, and 43 of the matched controls surviving to termination of the study. In spite of these early deaths, all male rats except one low-dose animal and one high-dose animal developed tumors, and among the females, 23 low-dose and 22 mid-dose animals developed tumors. Time-adjusted analysis of the incidence of tumors was performed in the female rats.

In male rats, there was a positive dose-related trend (P<0.001) in the incidence of angiosarcoma of the liver; furthermore, the incidences in the mid- and high-dose groups, but not that in the low-dose, were significantly higher (P<0.001, both groups) than that in the controls (controls 0/24, low-dose 5/24, mid-dose 11/24, high-dose 13/24). In females, the incidences in both the low- and mid-dose groups, but not that in the high-dose, were significantly higher (P = 0.002 and P = 0.005, respectively) than that in the controls (controls 0/24, low-dose 8/24, mid- dose 7/24, high-dose 2/9). Metastatic angiosarcomas were present in the lungs from a few of the rats in all three treated groups of both sexes.

In both male and female rats, there was a positive dose-related trend in the combined incidence of hepatocellular carcinoma and adenoma of the liver (males, P = 0.003; females, P<0.001); furthermore, the combined incidence of these tumors in the high-dose females, but not those in the low- and mid-dose, was significantly higher (P<0.001) than that in the controls (controls 0/24, low-dose 5/24, mid-dose 1/24, high-dose 7/9). The P-value of the combined incidence in the high-dose males (P = 0.025) is above the 0.016 level required by the Bonferroni inequality criterion, when multiple comparison is considered (controls 0/24, low-dose 0/24, mid-dose 3/24, high-dose 5/24). Nodular hyperplasia was observed in additional animals of each treated group of each sex. Thus, lasiocarpine was associated with proliferative lesions of hepatocytes as well as with angiosarcomas arising from endothelial cells of the liver.

The combined incidence of lymphoma or leukemia was significant in both the low- and mid-dose female groups (P≤ 0.018), but not in the high-dose group, perhaps because of the early deaths in this group (controls 2/24, low-dose 9/24, mid-dose 11/24, high-dose 1/23). The incidences of these tumors in the males were not significant.

It is concluded that under the conditions of this bioassay, lasiocarpine was carcinogenic in Fischer 344 rats producing hepatocellular tumors and angiosarcomas of the liver in both sexes and hematopoietic tumors in female animals.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive

TR-40 Bioassay of Hexachlorophene for Possible Carcinogenicity (CAS No. 70-30-4)

Hexachlorophene is a chlorinated bisphenol which was widely used as an antiseptic prior to 1972. It is highly effective against gram-positive bacteria and many pathogenic fungi.

A bioassay of hexachlorophene for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats.

Groups of 24 rats of each sex were administered hexachlorophene at one of three doses, either 17, 50, or 150 ppm, for 105-106 weeks. Higher doses of 200-600 ppm, used in 8-week subchronic studies, induced neuronal necrosis of the brain and clinical signs of toxicity. Matched-control groups consisted of 24 untreated rats of each sex. All surviving animals were killed at 105-106 weeks.

Mean body weights of the rats were unaffected by the hexachlorophene, and no clinical signs of toxicity were recorded. Survival also was unaffected, and adequate numbers of animals survived, permitting meaningful evaluation of the incidences of late-appearing tumors.

No tumors were present in a statistically significant incidence at any site in the treated rats.

It is concluded that under the conditions of this bioassay, hexachlorophene did not induce malignant or benign tumors in Fischer 344 rats.

Synonym: 2,2'-methylenebis(3,4,6-trichlorophenol)

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative

TR-41 Bioassay of Chlorothalonil for Possible Carcinogenicity (CAS No. 1897-45-6)

Chlorothalonil is a broad-spectrum fungicide which has been in use in the United States since 1963. It is registered for foliar and root applications on vegetables, fruits, green house plants, and turf, and as a seed treatment for cotton. Chlorothalonil is also used in formulating paints and stains for mildew resistance.

A bioassay of technical-grade chlorothalonil for possible carcinogenicity was conducted by administering the test chemical in the diet to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered chlorothalonil at one of two doses for 80 weeks, then observed for 30-31 weeks. Time-weighted average doses for both males and females were 5,063 or 10,126 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups combined with 55 untreated male or female rats from similar bioassays of five other test chemicals. All surviving rats were killed at 110-111 weeks.

Groups of 50 mice of each sex were administered chlorothalonil at one of two doses for 80 weeks, then observed for 11-12 weeks. Time-weighted average doses for males were 2,688 or 5,375 ppm, and for females, 3,000 or 6,000 ppm. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups combined with 50 untreated male or female mice from similar bioassays of five other test chemicals. All surviving mice were killed at 91-92 weeks.

Clinical signs that appeared with increasing frequency in dosed rats included hematuria and, from week 72 until termination of the study, bright-yellow urine. Since the dosed female mice did not have depression in mean body weights or decreased survival compared with the controls, they may have been able to tolerate a higher dose.

In rats, adenomas and carcinomas of the renal tubular epithelium occurred with a significant dose-related trend in both the males ($P = 0.030$) and the females ($P = 0.007$). These neoplasms also occurred at a higher incidence in the high-dose males ($P = 0.035$) and the high-dose females ($P = 0.016$) than in the corresponding controls (males: pooled controls 0/62, low-dose 3/46, high-dose 4/49; females: pooled controls 0/62, low-dose 1/48, high-dose 5/50). These tumors included both adenomas and carcinomas which are considered to be histogenically related. Thus these findings are interpreted as sufficient evidence for the carcinogenicity of chlorothalonil.

In mice, no tumors were found to occur at a greater incidence among dosed animals than among controls.

It is concluded that under the conditions of this bioassay, technical-grade chlorothalonil was carcinogenic to Osborne-Mendel rats, producing tumors of the kidney. Chlorothalonil was not carcinogenic for $B6C3F_1$ mice.

Synonym: 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-42 Bioassay of 5-Azacytidine for Possible Carcinogenicity (CAS No. 320-67-2)

5-Azacytidine, a synthetic analogue of cytidine, has been used as an investigational anticancer drug in the United States since 1970.

A bioassay of 5-azacytidine for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered 5-azacytidine at one of two doses, either 2.6 or 5.2 mg/kg body weight, in buffered saline three times per week for 34 weeks, and were then observed for 46 or 47 weeks. Controls consisted of groups of 15 rats of each sex that received injections of buffered saline (vehicle controls) and 15 rats of each sex that were untreated (untreated controls). All surviving rats were killed at 80 or 81 weeks.

Groups of 35 mice of each sex were administered the chemical at one of two doses, either 2.2 or 4.4 mg/kg body weight, in buffered saline three times per week for 52 weeks, and were then observed for 29 or 30 weeks. Controls consisted of groups of 15 mice of each sex that received injections of buffered saline (vehicle controls) and 15 mice of each sex that were untreated (untreated controls). All surviving mice were killed at 81 or 82 weeks.

5-Azacytidine was toxic to the animals in this bioassay, since mean body weights of both treated rats and treated mice were lower than those of the corresponding vehicle controls, and since none of the high-dose male and female rats and high-dose female mice lived to the end of the bioassay. In treated male and female rats and male mice, survival was inadequate for meaningful statistical analyses of the incidences of tumors.

Only one male and three female high-dose rats had tumors, and none of the tumors in the low-dose group of either sex were present at a significantly increased incidence using any of the statistical tests. Bone-marrow atrophy was present in both treated groups of both sexes of rats.

Only five high-dose male mice and one high-dose female mouse had neoplasms. In low-dose female mice, however, lymphocytic and granulocytic neoplasms of the hematopoietic system occurred in 17 animals, even though only 54% survived until week 81. Granulocytic neoplasms were observed in 10/29 low-dose female mice, but in no other group, and were significant ($P = 0.010$) compared with the vehicle controls. The incidence of combined lymphoma and granulocytic neoplasms was highly significant in the low-dose females (vehicle controls 0/14, low-dose 17/29, $P < 0.001$). No tumors were observed at a significant incidence in male mice. Bone-marrow atrophy was present in high-dose female mice.

It is concluded that under the conditions of this bioassay, the short life span and short duration of treatment of Sprague-Dawley rats of either sex and of male $B6C3F_1$ mice precluded evaluation of the carcinogenicity of 5-azacytidine in these groups; however, the induction of tumors of the hematopoietic system in female $B6C3F_1$ mice was associated with the administration of 5-azacytidine.

Synonym: 4-amino-1-β-D-ribofuranosyl-1,3,5-triazine-2(1H)-one

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Inadequate Study
Female Mice:	Positive

TR-43 Bioassay of Emetine for Possible Carcinogenicity (CAS No. 483-18-1)*

A bioassay of emetine, an amebicide and anticancer drug, for possible carcinogenicity was conducted by administering the test material by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered emetine at one of two doses, either 0.5 or 1 mg/kg body weight, three times per week for 52 weeks, and then observed for an additional 31 or 32 weeks. Control groups of each sex consisted of 10 untreated rats (untreated controls) and 10 rats injected with buffered saline (vehicle controls). Pooled-control groups, used for statistical evaluation, consisted of the vehicle-control rats of each sex for this study combined with 15 vehicle-control rats of each sex from a similar bioassay of another test chemical. All surviving rats were killed at 83 or 84 weeks.

Initially, groups of 35 mice of each sex were administered emetine at one of two doses, either 3.2 or 6.4 mg/kg body weight (mid- and high-dose), three times per week. Control groups of each sex consisted of 15 untreated mice (untreated controls) and 15 mice injected with buffered saline (vehicle controls). Due to high mortality rates in the initial treated groups, additional groups of 35 mice of each sex were later put on study at 1.6 mg/kg (low-dose), together with 10 untreated-control and 10 vehicle-control mice of each sex. The high-dose males were treated for 28 weeks and the mid- and high-dose females for 40 and 33 weeks, respectively. Mid- and low-dose male mice and low-dose female mice were treated for 52 weeks, and then observed for an additional 20 or 26 weeks. All surviving mice were killed at 78-83 weeks.

Emetine was toxic to male rats at the high dose, to both sexes of mice at the high and mid doses and to a lesser extent at the low dose, as shown by the low survival in these groups. Twenty-six percent of the high-dose male rats and 69% of the high-dose female rats, but none of the high- and mid-dose mice of either sex, survived to the end of the study. In the low-dose mice, 30/35 males and 21/35 females lived at least 1 year, and the median time on study was 72 weeks for males and 59 weeks for females.

No tumors occurred at a statistically significant incidence in treated rats or mice compared with controls; however, it should be noted that in this study, treatment of both species was stopped at week 52 and the studies were terminated by week 83, which is earlier than in current bioassays where animals are treated until termination of the studies at 2 years. In addition, there was poor survival among the treated mice.

It is concluded that the results of this study do not allow evaluation of the possible carcinogenicity of emetine.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Inadequate Study
Female Mice:	Inadequate Study

*The technical report states that Emetine Hydrochloride (CAS No. 316-42-7) was the actual chemical tested rather than Emetine in its pure form; therefore, the CAS number for Emetine Hydrochloride is used to track this study in the NTP CHEMTRACK database.

TR-44 In Vitro Carcinogenesis: Guide to the Literature, Recent Advances and Laboratory Procedures

Note to the Reader: This document is the published proceedings of a seminar and workshop held in July, 1976 and sponsored by the National Cancer Institute.

Report Date: 1978

TR-45 Bioassay of Chlorpropamide for Possible Carcinogenicity (CAS No. 94-20-2)

Chlorpropamide is an oral hypoglycemic agent of the arylsulfonylurea type. Chlorpropamide was selected for testing in the carcinogenesis program because it is used extensively and for prolonged periods in humans.

A bioassay of chlorpropamide for possible carcinogenicity was conducted by administering the test material in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered chlorpropamide as follows: rats 5 days per week for 103 to 105 weeks at 3,000 or 6,000 ppm, and mice 5 days per week for 34 weeks at 5,000 or 10,000 ppm, followed by 70 weeks at 2,500 or 5,000 ppm. The time-weighted average doses for mice were 3,317 ppm for low-dose males and females, and 6,635 ppm for high-dose males and females. Matched controls consisted of groups of 15 untreated rats and 15 untreated mice of each sex. All surviving rats and mice were killed at 103 to 105 weeks.

Mean body weights of both low- and high-dose rats were lower than those of the matched controls throughout the study. In mice, doses were reduced at week 34, due to early deaths in the high-dose groups; following this adjustment the treated mice gained weight, but the weights never reached those of the controls. Survival of the treated rats and the low-dose mice was adequate for meaningful statistical analyses of the incidences of tumors.

In both rats and mice, the incidences of tumors among the treated groups were not significantly increased in comparison with matched controls.

It is concluded that under the conditions of this bioassay, chlorpropamide was not carcinogenic for Fischer 344 rats or $B6C3F_1$ mice.

Synonym: 1-[(p-chlorophenyl)sulfonyl]-3-propylurea

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-46 Bioassay of Ethionamide for Possible Carcinogenicity (CAS No. 536-33-4)

A bioassay of the chemotherapeutic drug ethionamide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 34 or 35 mice of each sex were administered ethionamide at one of the following doses, either 1,500 or 3,000 ppm for the rats and either 1,000 or 2,000 ppm for the mice. The animals were treated 5 days per week for 78 weeks, then observed for an additional 25 or 26 weeks. Matched controls consisted of groups of 15 untreated rats and 15 untreated mice of each sex. All surviving animals were killed at 103 or 104 weeks.

Mean body weights of the treated rats and mice were lower than those of the corresponding matched controls during most or all of the study. Survival in the rats was sufficient to allow development of late-appearing tumors. In the mice, survival of the high-dose males (27%), matched-control males (7%), and low-dose females (37%) to the end of the study was low, and the deaths were associated with suppurative lung lesions. However, tests for dose-related trend in mortality were not significant in either sex, and 47% or more of all groups of mice except control males were alive at 78 weeks.

In the rats, a variety of neoplasms were observed in treated and control groups of each sex. The lesions were of types commonly found in Fischer 344 rats, and none of the incidences of tumors in dosed animals were statistically significant when compared with controls.

In the mice, the incidences of malignant lymphoma were slightly higher in dosed than in control mice (males: controls 2/15, low-dose 8/34, high-dose 4/34; females: controls 2/15, low-dose 4/31, high-dose 10/34). The incidences were not significant by any of the statistical tests used, including the Tarone and Cox tests using the life-table method.

It is concluded that under the conditions of this bioassay, ethionamide was not carcinogenic in either Fischer 344 rats or $B6C3F_1$ mice.

Synonym: 2-ethylthioisonicotinamide

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-47 Bioassay of 4,4'-Thiodianiline for Possible Carcinogenicity (CAS No. 139-65-1)

4,4'-Thiodianiline is an intermediate in the manufacture of several diazo dyes.

A bioassay of 4,4'-thiodianiline for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered 4,4'-thiodianiline 5 days per week at one of the following doses, either 1,500 or 3,000 ppm for the rats and either 2,500 or 5,000 ppm for the mice. The period of administration of the chemical was 68-72 weeks for the rats and 77 or 79 weeks for the mice, depending on the length of survival time of the animals. Matched controls consisted of groups of 15 untreated rats and 14 untreated mice of each sex. All surviving matched-control rats were killed at 104 weeks; all surviving matched-control mice were killed at 91 weeks.

The administration of 4,4'-thiodianiline resulted in marked reduction in mean body weights of the rats and mice of each sex, and all dosed animals died prior to the scheduled end of the study.

Tumors of epithelial origin were found in many organs, and all dosed rats except one were affected at one or more sites (males: skin, ear canal, lungs, liver, colon, and thyroid; females: ear canal, lung, liver, thyroid, and uterus). These tumors were not found among any of the matched-control animals.

In male rats, several of these neoplastic lesions occurred with statistically significant incidences in one or both of the dosed groups. The incidences of hepatocellular carcinoma (controls 0/15, low-dose 21/33, high-dose 10/33) and of follicular-cell carcinoma of the thyroid (controls 0/15, low-dose 28/33, high-dose 32/33) were significant in each of the groups at $P \leq 0.014$. The combined incidences of squamous-cell carcinoma and squamous-cell papilloma of the ear canal in the low- and high-dose groups of males were both significantly higher (low-dose $P = 0.001$, high-dose $P = 0.037$) than that in the control group (controls 0/15, low-dose 15/33, high dose 8/33). The first such tumor in the high-dose group was observed at 25 weeks.

Also in low-dose male rats, squamous-cell papilloma of the skin occurred in 4/33 animals, and squamous-cell carcinoma of the skin in 1/33, but no tumors of either type occurred in the controls. The incidences of these lesions were too low to have statistical significance. The majority of the squamous-cell tumors of the skin were located in one area near the commissure of the mouth. Only one such tumor occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the skin may be associated with administration of the chemical. Adenocarcinoma of the colon occurred in six low-dose male rats and in one high-dose male rat, but not in any of the controls. This incidence is not statistically significant; however, no such tumors occurred among the 235 historical-control male rats at this laboratory; thus, the tumors of the colon are considered to be related to administration of 4,4'-thiodianiline.

In female rats, the incidences of hepatocellular adenoma or carcinoma in the dosed groups were greater than those in the controls, but not statistically significant (controls 0/15, low-dose 6/32, high-dose 3/33). Follicular-cell carcinoma of the thyroid and adenocarcinoma of the uterus occurred in the females administered the test chemical at statistically significant incidences ($P < 0.001$) in both dosed groups (follicular-cell carcinoma: controls 0/14, low-dose 24/33, high-dose 32/32; adenocarcinoma: controls 0/15, low-dose 31/33, high-dose 23/32). Squamous-cell papilloma or carcinoma of the ear canal occurred at increased, but not statistically significant, incidences in female rats (controls 0/15, low-dose 6/33, high-dose 3/33). However, no such tumors occurred among the 235 historical-control female rats at this laboratory; thus, the tumors of the ear canal are considered to be related to administration of the chemical.

In mice of each sex, the incidence of hepatocellular carcinoma was statistically significant ($P < 0.001$) in each of the dosed groups (males: controls 1/13, low-dose 32/34, high-dose 22/24, females: controls 0/12, low-dose 32/34, high-dose 30/31). In the males, follicular-cell carcinoma of the thyroid occurred at statistically significant incidences ($P \leq 0.001$) in both the low- and high-dose groups (controls 0/14, low-dose 15/33, high-dose 20/23). In the females, the incidence was significant ($P = 0.002$) only at the high dose (controls 0/11, high-dose 15/30); however, when follicular-cell adenoma and carcinoma were combined, the incidences in both the low- and high-dose groups of females were significantly higher (low-dose $P = 0.025$, high-dose $P < 0.001$) than that in the control group (controls 0/11, low-dose 11/33, high-dose 18/30).

It is concluded that under the conditions of this bioassay, 4,4'-thiodianiline was carcinogenic for Fischer 344 rats, inducing tumors in the liver, thyroid, colon, and ear canal of male rats, and the thyroid, uterus, and ear canal of female rats. 4,4'-Thiodianiline was carcinogenic for $B6C3F_1$ mice, inducing tumors in the liver and thyroid of both males and females.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-48 Bioassay of Pyrazinamide for Possible Carcinogenicity (CAS No. 98-96-4)

A bioassay of the tuberculostatic drug pyrazinamide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered pyrazinamide at one of two doses, either 5,000 or 10,000 ppm, for 78 weeks, and then observed for an additional 26 or 27 weeks. Matched controls consisted of groups of 15 untreated rats and 15 untreated mice of each sex. High-dose male mice died or were killed by week 92; all other surviving animals were killed at weeks 104 or 105.

Mean body weights of the dosed male rats were slightly lower than those of the matched controls, while mean body weights of the dosed females were more nearly comparable to those of the controls. A sufficient number of rats in each group was at risk to termination of the study at weeks 104-105 for the development of late-appearing tumors.

In mice, administration of pyrazinamide had no consistent effect on mean body weights. Survival to termination of the study was low, particularly among the control groups.

In rats, no lesions could clearly be related to administration of the chemical.

In mice, interstitial and suppurative myocarditis in the dosed animals and suppurative bronchopneumonias in both dosed and matched control mice of each sex were associated with increased deaths. In the females, there was a significant positive dose-related trend ($P = 0.037$) in the incidence of lymphoma (matched controls 0/13, low-dose 2/25, high-dose 6/29); however, the incidences in each of the dosed groups were not significant when compared with that in the matched controls. In addition, the poor survival and the small size of the control group precluded making a clear association of the incidence of these tumors with administration of the chemical.

It is concluded that under the conditions of this bioassay, the early deaths and small size of the control group precluded a conclusion regarding the carcinogenicity of pyrazinamide in female $B6C3F_1$ mice. Pyrazinamide was not carcinogenic for Fischer 344 rats or for male mice.

Synonym: pyrazinecarboxamide

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Inadequate Study

TR-49 Bioassay of Acronycine for Possible Carcinogenicity (CAS No. 7008-42-6)

Acronycine, an alkaloid derived from the bark of the Australian scrub ash, has been investigated as an experimental anticancer drug. Acronycine was selected for screening in the carcinogenesis program in an attempt to evaluate the carcinogenicity of certain drugs that may be used for prolonged periods in humans.

A bioassay of acronycine for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Initially, groups of 35 rats of each sex were administered acronycine at one of two doses, either 7.5 or 15 mg/kg body weight, in a vehicle composed of 0.05% polysorbate 80 in phosphate-buffered saline. Control groups of each sex consisted of 10 untreated rats (untreated controls) and 10 rats injected with the vehicle (vehicle controls). Because of high mortality rates in the dosed animals, new dosed groups of 35 rats of each sex were started later at a dose of 3.75 mg/kg. Additional groups of 10 untreated and 10 vehicle controls of each sex were also started. The rats were administered the acronycine or the vehicle for 51 or 52 weeks, then observed for an additional 28-30 weeks. All surviving rats were killed at 80-82 weeks.

Initially, groups of 35 mice of each sex were administered acronycine at one of two doses, either 12.5 or 25 mg/kg body weight, in a vehicle composed of 0.05% polysorbate 80 in phosphate-buffered saline. Control groups of each sex consisted of 10 untreated mice (untreated controls) and 10 mice injected with the vehicle (vehicle controls). Because of high mortality rates in the dosed animals, two additional dosed groups were started later: 35 mice of each sex at 6 mg/kg and 40 mice of each sex at 2 mg/kg, together with 10 untreated controls and 10 vehicle controls of each sex for the groups dosed at 6 mg/kg, and 20 untreated controls and 20 vehicle controls for the groups dosed at 2 mg/kg. Periods of administration of the chemical to the mice varied from 25 weeks to 92 weeks, depending on toxicity or length of time of survival. Surviving control animals were killed at 78-105 weeks.

Acronycine was toxic to rats and mice of each sex at the doses used in this bioassay, as shown by the high mortality rates in all but the low-dose groups and by the lower mean body weights in dosed rats and mice at all doses throughout most of the bioassay.

Because of this high number of deaths, time-adjusted statistics are used for the analyses of all incidences of tumors.In male rats, the dose-related trend in the mid- and high-dose groups for the incidence of osteosarcoma at all sites was significant ($P = 0.002$) using the respective vehicle-control group (vehicle controls 0/8, mid-dose 13/30, high-dose 12/18). Comparisons of the individual groups with respective control groups were also significant for the mid-dose ($P = 0.022$) and high-dose

($P = 0.002$) groups, but not for the low-dose group. In female rats, osteosarcoma was observed only in 1/8 high-dose animals.

Sarcomas and other related tumors of the peritoneum were observed in all three dosed groups of both male and female rats, but in none of the control groups (males: low-dose 5/30, mid-dose 3/26, high-dose 7/16; females: low-dose 1/35, mid-dose 5/30, high-dose 13/28). In both sexes, the dose-related trends were significant (males, $P = 0.006$; females, $P = 0.002$), and the comparison of the incidences in the high-dose females with the vehicle-control group was significant ($P = 0.016$). None of the incidences in the individual dosed groups of males were significant when compared with vehicle controls. However, since the tumors were observed in all dosed groups but did not occur in historical-control animals at this laboratory, they are considered to be related to the administration of the chemical.

In female rats, the incidence of all tumors of epithelial origin of the mammary gland was significant only at the low dose (low-dose vehicle controls 1/10, low-dose 22/35, $P = 0.004$). Adenocarcinomas of the mammary gland were observed in seven low-dose, five mid-dose, and two high-dose female rats, but in no control females. The reverse dose relationship of both benign and malignant tumors was probably due to the higher number of early deaths which occurred in the high-dose group.

In mice, the low survival in all dosed groups except the low-dose animals precluded an evaluation of the significance of the incidences of tumors. Lymphomas occurred in low-dose groups of both males and females; however, the incidence of lymphoma in different control groups was highly variable. The high incidence in the low-dose vehicle controls may have been due to a procedural problem associated with the possibility of transfer of tumor cells or oncogenic viruses during the intraperitoneal injection of the test chemical.

It is concluded that under the conditions of this bioassay, the low survival of the dosed and control mice and the possible procedural problems associated with the intraperitoneal injection of the chemical did not allow a determination to be made of the carcinogenicity of acronycine in this species.

In Sprague-Dawley rats, acronycine in the vehicle of 0.05% polysorbate 80 in phosphate-buffered saline was carcinogenic, producing tumors of the mammary gland in females, osteosarcomas in males, and sarcomas and other related tumors of the peritoneum in both males and females.

Synonym: 3,12-dihydro-6-methoxy-3,3,12-trimethyl-7H-pyrano(2,3-c)acridin-7-one

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Inadequate Study
Female Mice:	Inadequate Study

TR-50 Bioassay of Acetohexamide for Possible Carcinogenicity (CAS No. 968-81-0)

Acetohexamide is an oral hypoglycemic agent of the arylsulfonyl-urea group with a potency between that of tolbutamide and chlorpropamide.

A bioassay of acetohexamide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered acetohexamide in the diet at one of two doses, either 10,000 or 20,000 ppm, for 103 weeks and then observed for 2 to 4 additional weeks. Matched controls consisted of 15 untreated rats of each sex. All surviving rats were killed at 105 to 107 weeks.

Groups of 35 mice of each sex were administered acetohexamide at one of two doses for 103 weeks and then observed for 4 or 5 additional weeks. Time-weighted average doses were 6,359 or 12,718 ppm. Matched controls consisted of 15 untreated mice of each sex. All surviving mice were killed at 107 or 108 weeks.

Mean body weights of the dosed rats and mice of both sexes were lower than those of the corresponding matched controls throughout the study, and the depressions in weight were dose related. Except for the female mice, sufficient numbers of animals survived long enough to be at risk for development of late-appearing tumors.

In the rats, the only tumor occurring with greater incidence in dosed than in matched-control animals was leukemia (males: matched controls 0/15, low-dose 10/35, high-dose 4/35; females: matched controls 0/14, low-dose 7/35, high-dose 4/34). Only the incidence in the low-dose males was statistically significant ($P = 0.018$). All of these animals had undifferentiated (mononuclear cell) leukemia, which commonly occurs spontaneously in Fischer 344 rats, except for two with lymphocytic leukemia. The incidence of combined leukemia and lymphoma in historical-control male rats at this laboratory in the bioassay program to date is 24/235 (10.2%), which is higher than that in the matched controls. Thus, the incidence in the low-dose males cannot be clearly associated with administration of the test chemical.

In the mice, the only neoplasms that occurred at a higher incidence in dosed groups than in matched controls were lymphomas in the males, but the incidences were not statistically significant (matched controls 1/15, low-dose 9/35, high-dose 3/34). These types of lesions are found commonly in untreated $B6C3F_1$ mice. The incidence of lymphomas in the historical-control male mice is 28/536 (5.2%).

It is concluded that under the conditions of this bioassay, acetohexamide was not carcinogenic for either Fischer 344 rats or $B6C3F_1$ mice.

Synonym: 1-[(p-acetylphenyl)-sulfonyl]-3-cyclohexyl-urea

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-51 Bioassay of Tolazamide for Possible Carcinogenicity (CAS No. 1156-19-0)

Tolzamide is an oral hypoglycemic agent of the arylsulfonylurea type, similar to tolbutamide, chlorpropamide, and acetohexamide.

A bioassay of the hypoglycemic drug tolazamide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered tolazamide at one of two doses, either 5,000 or 10,000 ppm, for 103 weeks. Matched controls consisted of 15 rats and 15 mice of each sex. All surviving rats and mice were killed at 104 or 105 weeks.

Survival rates for the dosed rats of each sex were higher than those for the matched controls, and were adequate for the development of late-appearing tumors. Survival rates for the mice were lower than those for the rats, particularly for the dosed females (matched controls 67%, low-dose 34%, high-dose 32%). However, a large number of these deaths in the dosed females occurred after 90 weeks on study, and survival of both males and females was adequate for the development of late-appearing tumors.

All observed tumors were of types commonly found in the strains of animals used, and there were no statistically significant increases in the incidence of tumors in the dosed animals as compared with controls.

It is concluded that under the conditions of this bioassay, tolazamide was not carcinogenic for Fischer 344 rats or $B6C3F_1$ mice.

Synonym: N-(p-toluenesulfonyl)-N'-hexamethyleniminourea

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-52 Bioassay of 3-Nitropropionic Acid for Possible Carcinogenicity (CAS No. 504-88-1)

3-Nitropropionic acid was selected for testing for carcinogenic activity because it was known to demonstrate varying degrees of toxicity in man and animals, and because its use in food preparations and its identification as a contaminant in foods suggested there was a possibility of long-term human exposure.

A bioassay of 3-nitropropionic acid (95% pure) for possible carcinogenicity was conducted by administering the test chemical by gavage to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered 3-nitropropionic acid at one of the following doses by gavage 5 days per week. For male rats, the doses were 0.425 or 0.85 mg/animal/day; for females, they were 0.6 or 1.2 mg/animal/day. For both sexes of mice, the doses were 0.375 or 0.75 mg/animal/day. The rats were administered the chemical for 110 weeks and the mice for 104 weeks. The controls consisted of 50 untreated rats and 50 untreated mice of each sex. All surviving rats were killed at 111 weeks and all surviving mice at 104 or 105 weeks.

Mean body weights and mortality of the dosed animals were not markedly affected by 3-nitropropionic acid under the conditions of this bioassay, indicating that the maximum tolerated dose may not have been reached. The various clinical signs observed were common to both dosed and control groups.

In rats, the combination of neoplastic nodule of the liver and hepatocellular carcinoma occurred in the males with a significant dose-related trend ($P = 0.010$) and with a higher incidence ($P = 0.012$) in the high-dose group of animals than in the controls (controls 0/49, low-dose 3/50, high-dose 6/49). All but one of these tumors were neoplastic nodules. In the females, only two neoplastic nodules occurred, one in each of the dosed groups. Biliary hyperplasia occurred at a higher incidence in the dosed males than in the corresponding controls (controls 19/50, low-dose 32/50, high-dose 36/50), but the incidence of this lesion in the dosed females was not increased as compared with controls. There was also a dose-related trend ($P = 0.033$) in the incidence of pancreatic islet-cell adenoma in the male rats (controls 4/49, low-dose 6/50, high-dose 11/50); however, direct comparisons of incidences in the dosed and control groups were not statistically significant. The historical incidence of pancreatic islet-cell adenoma among 100 control Fischer 344 rats at the laboratory was 7/100 (7%). In addition, focal myocardial fibrosis was observed at a higher incidence in dosed rats than among controls (males: controls 1/4, low-dose 17/49, high-dose 24/48; females: controls 2/48, low-dose 9/46, high-dose 9/50).

In mice, each type of neoplasm found in the dosed and control mice has been encountered previously as a spontaneous lesion. No specific tumor was found to occur at a statistically significantly higher incidence among dosed mice than among the respective control groups.

It is concluded that under the conditions of this bioassay, there was an elevated incidence of hepatocellular neoplasms, primarily benign, and of islet-cell adenomas of the pancreas in male Fischer 344 rats receiving 3-nitropropionic acid as compared with controls; however, there was no conclusive evidence that 3-nitropro-

pionic acid was carcinogenic in these animals. The chemical was not carcinogenic in female rats or in male or female B6C3F$_1$ mice.

Synonyms: β-nitropropionic acid; hiptagenic acid

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-53 Bioassay of 2-Amino-5-Nitrothiazole for Possible Carcinogenicity (CAS No. 121-66-4)

2-Amino-5-nitrothiazole is an antiprotozoal drug for animals which is now used in the form of the acetyl derivative to control histomoniasis (blackhead) in turkeys. The use of acetyl-2-amino-5-nitrothiazole in animal feed and the allowable residues in food products from treated animals (0.1 ppm) are regulated by the Food and Drug Administration.

A bioassay of 2-amino-5-nitrothiazole for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were fed 2-amino-5-nitrothiazole at one of the following doses, either 300 or 600 ppm for rats, and either 50 or 100 ppm for mice. The rats were dosed for 110 weeks, followed by 1 week of observation; the mice were dosed for 104 weeks. Matched controls consisted of 50 untreated rats and 50 untreated mice of each sex. All surviving rats were killed at week 111, all surviving mice at week 104.

The mean body weights of the groups of rats and mice fed 2-amino-5-nitrothiazole in the diet were slightly lower than those of the controls throughout most of the period of administration. No other clinical signs related to administration of the chemical were noted. There was a dose-related trend in mortality only in the male rats; however, sufficient numbers of rats were at risk in all groups for development of late-appearing tumors.

In male rats, there was a significant dose-related trend ($P = 0.044$) in the incidences of malignant lymphomas, lymphocytic leukemias, or undifferentiated leukemias, although the results of direct comparisons of incidences in each of the dosed groups with those in the controls were not significant. There was also a significant dose-related trend in the incidence of granulocytic leukemia in the male rats ($P = 0.014$) and a significantly increased incidence of this tumor ($P = 0.023$) in the high-dose group (matched controls 2/50, low-dose 4/50, high-dose 9/49). When the incidences of all neoplasms of the hematopoietic system lymphomas and all leukemias) were combined, greater significance was attained for both the dose-related trend ($P = 0.001$) and the direct comparison ($P = 0.002$) of the incidence of the high-dose group with that in the matched controls (controls 13/50, low-dose 9/50, high-dose 28/49). The reliability of the incidence of hematopoietic tumors in the male controls was supported by that for male controls observed in a similar bioassay of another test chemical at the same laboratory (13/50). The incidences of the combined hematopoietic tumors in the dosed female rats were not significant when compared with the incidence in the matched controls.

In female rats, there was a significant dose-related trend in the incidence of chromophobe adenomas of the pituitary ($P = 0.016$) and a higher incidence ($P = 0.021$) in the high-dose group than in the matched controls (controls 19/45, low-dose 29/47, high-dose 29/44). The incidence of this lesion in dosed male rats was much lower than that in dosed females, and the dose-related trend ($P = 0.048$) was only marginally significant (controls 3/46, low-dose 3/45, high-dose 8/43). The incidences of chromophobe adenomas of the pituitary which were observed in control groups of rats used in a similar bioassay of another test chemical at the same laboratory were 13/49 (27%) for the males and 26/50 (52%) for the females. Because of the variability in incidences of the tumor among different control groups, the occurrence of chromophobe adenomas of the pituitary in the dosed female rats cannot be clearly associated with the administration of 2-amino-5-nitrothiazole.

Also in female rats, there was a higher incidence of endometrial stromal polyps of the uterus in the low-dose group ($P = 0.023$) than in the matched controls (controls 2/50, low-dose 9/49, high-dose 3/50). Since, however, only three high-dose animals had this tumor, the occurrence of uterine tumors in the low-dose group cannot be clearly associated with administration of the test chemical.

In the mice, no neoplasms were observed at a statistically significant incidence in the dosed groups when compared with the controls.

It is concluded that under the conditions of this bioassay, the occurrence of tumors of the hematopoietic system, i.e., lymphoma and granulocytic leukemia, in dosed male Fischer 344 rats was associated with administration of 2-amino-5-nitrothiazole. 2-Amino-5-nitrothiazole was not carcinogenic in female Fischer 344 rats or in male or female B6C3F$_1$ mice.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-54 Bioassay of 2,4-Dinitrotoluene for Possible Carcinogenicity (CAS No. 121-14-2)

2,4-Dinitrotoluene, a precursor in the synthesis of azo dyes, was selected for bioassay by the National Cancer

Institute along with other dye intermediates in an attempt to elucidate those chemicals which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry. 2,4-Dinitrotoluene is used by the munitions industry as a modifier for smokeless powders and, to a limited extent, as a gelatinizing and waterproofing agent in military and commercial explosive compositions.

A bioassay of practical-grade 2,4-dinitrotoluene for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. 2,4-Dinitrotoluene was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. For male and female rats, the high and low time-weighted average dietary concentrations of 2,4-dinitrotoluene were 0.02 and 0.008 percent, respectively. For male and female mice, the high and low time-weighted average concentrations were 0.04 and 0.008 percent, respectively. After a 78-week period of compound administration, observation of the rats continued for an additional 26 weeks and observation of the mice continued for 13 additional weeks.

For the chronic rat bioassay, 25 rats of each sex were placed on test as high dose controls, and 50 rats of each sex served as the low dose controls. For the mice, 50 males and 50 females were placed on test as controls for each of the high dose and low dose groups.

In both species the survival in all groups was adequate for statistical analysis of late-appearing tumors.

In the male rats, a significantly increased incidence of fibroma of the skin and subcutaneous tissue occurred in both the high and the low dose groups when compared to their respective controls. A statistically significant incidence of fibroadenoma of the mammary gland occurred in the high dose female rats.

Among the mice a variety of tumors was observed but none were considered to be associated with the dietary administration of 2,4-dinitrotoluene.

Under the conditions of this bioassay dietary administration of 2,4-dinitrotoluene to Fischer 344 rats induced benign tumors (i.e., fibroma of the skin and subcutaneous tissue in males and fibroadenoma of the mammary gland in females). No evidence was provided for the carcinogenicity of the compound in $B6C3F_1$ mice of either sex.

Synonyms: 1-methyl-2,4-dinitrobenzene; 2,4-dinitrotoluol; 2,4-DNT

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-55 Bioassay of 1,2-Dichloroethane for Possible Carcinogenicity (CAS No. 107-06-2)

1,2-Dichloroethane, a chlorinated aliphatic hydrocarbon, is one of several halogenated solvents selected for bioassay by the National Cancer Institute. Although the major use of 1,2-dichloroethane is as an intermediate in the synthesis of vinyl chloride, the compound also finds application as a constituent in lead-containing antiknock preparations, as an ingredient in fumigant-insecticide formulations and, to a more limited extent, as a component of metal degreasing mixtures. 1,2-Dichloroethane is additionally employed as an intermediate in the synthesis of the chlorinated solvents 1,1,1-trichloroethane, trichloroethylene, and perchloroethylene and as a constituent of rubber cements and acrylic-type adhesive formulations.

A bioassay of technical-grade 1,2-dichloroethane for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. 1,2-Dichloroethane in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The 78-week period of chemical administration was followed by an observation period of 32 weeks for the low dose rats of both sexes. The last high dose male rat died after 23 weeks of observation and the last high dose female rat died after 15 weeks of observation. All treated groups of mice were observed for an additional 12 or 13 weeks following chemical administration.

Initial dosage levels for the chronic bioassay were selected on the basis of a preliminary subchronic toxicity test. Subsequent dosage adjustments were made during the course of the chronic bioassay. The time-weighted average high and low doses of 1,2-dichloroethane in the chronic study were 95 and 47 mg/kg/day, respectively, for rats of both sexes. The high and low time-weighted average doses for the male mice were 195 and 97 mg/kg/day, respectively, and 299 and 149 mg/kg/day, respectively, for the female mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil at the same times that dosed animals were gavaged with the 1,2-dichloroethane mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

A statistically significant positive association between dosage and the incidence of squamous-cell carcinomas of the forestomach and hemangiosarcomas of the circulatory system occurred in the male rats, but not in the females. There was also a significantly increased incidence of adenocarcinomas of the mammary gland in female rats.

The incidences of mammary adenocarcinomas in female mice were statistically significant. There was a statistically significant positive association between chemical administration and the combined incidences of endometrial stromal polyps and endometrial stromal

sarcomas in female mice. The incidence of alveolar/bronchiolar adenomas in both male and female mice was also statistically significant.

Under the conditions of this study, 1,2-dichloroethane was carcinogenic to Osborne-Mendel rats, causing squamous-cell carcinomas of the forestomach, hemangiosarcomas, and subcutaneous fibromas in male rats and causing mammary adenocarcinomas in female rats. This compound was also found to be carcinogenic to $B6C3F_1$ mice, causing mammary adenocarcinomas and endometrial tumors in female mice, and causing alveolar/bronchiolar adenomas in mice of both sexes.

Synonyms: ethylene chloride; ethylene dichloride; alpha beta dichloroethane

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-56 Bioassay of N,N'-Dicyclohexylthiourea for Possible Carcinogenicity (1212-29-9)

N,N'-dicyclohexylthiourea is a chemical intermediate used in the production of dicyclohexylcarbodimide, a reagent used in the synthesis of peptide and phosphodiester internucleotide bonds.

A bioassay of N,N'-dicyclohexylthiourea for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered N,N'-dicyclohexylthiourea at one of two doses, either 25,000 or 50,000 ppm, for 109 weeks for rats or 104 weeks for mice. Matched controls consisted of 50 untreated rats or 50 untreated mice of each sex.

Mean body weights of male rats and male mice were unaffected by the compound, whereas mean body weights of the females of each species showed mild dose-related retardation over the bioassay period, when compared with the matched controls. Survival was sufficient to termination of the study in all groups of both rats and mice for the development of late-appearing tumors.

In male rats there was an increased incidence of hyperplasia of the follicular cells of the thyroid (males: controls 3/43, low-dose 16/49, high-dose 15/49; females: controls 1/48, low-dose 7/48, high-dose 5/49). The incidences of tumors of the follicular cells of the thyroid, although increased among the dosed male rats, were not statistically significant in either sex.

In mice, a variety of neoplasms of the type usually encountered in the $B6C3F_1$ strain were observed in both dosed and control animals. None of the tumors occurred at statistically significant incidences. Follicular-cell hyperplasia of the thyroid was observed at an increased incidence in both the dosed males and females (males: controls 3/39, low-dose 12/46, high-dose 9/45; females: controls 8/38, low-dose 22/46, high-dose 21/46).

An increase in proliferative lesions of the follicular cells of the thyroid was associated with the administration of N,N'-dicyclohexylthiourea in both Fischer 344 rats and $B6C3F_1$ mice. However, because statistical significance was not achieved and because thyroid tumors are not rare, spontaneous lesions in these strains of animals and occur with a variable incidence, it is concluded that under the conditions of this bioassay, N,N'-dicyclohexylthiourea was not demonstrated to be carcinogenic in either species.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-57 Bioassay of β-TGdR for Possible Carcinogenicity (CAS No. 789-61-7)

Beta-2'-deoxy-6-thioguanosine monohydrate (β-TGdR) is an experimental anticancer drug and a derivative of the anticancer drug 6-thioguanine (6-TG).

A bioassay of beta-2'-deoxy-6-thioguanosine monohydrate (β-TGdR) for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered β-TGdR in a buffered saline and polysorbate 80 vehicle at one of two doses, either 3.5 or 7 mg/kg body weight, three times per week for 52 weeks, then observed for an additional 26 weeks. Controls consisted of groups of 10 rats of each sex, which were either administered the vehicle alone (matched vehicle controls) or were untreated (matched untreated controls). Pooled controls consisted of the matched vehicle controls of each sex from the current bioassay, combined with 20 corresponding vehicle controls of each sex from similar bioassays of two other test chemicals. All surviving rats were killed at 78 or 79 weeks.

Groups of 35 mice of each sex were administered the chemical in a buffered saline and polysorbate 80 vehicle at one of two doses, either 2 or 4 mg/kg, three times per week for 52 weeks, then observed for periods of up to 27 weeks, depending on length of survival. Because of severe toxicity at the high dose, resulting in loss of all mice by week 12 (males) or week 25 (females), additional groups of 35 mice of each sex were administered 1 mg/kg on the same schedule. Controls consisted of groups of 15 mice of each sex, which were either administered the vehicle or were untreated. Pooled controls consisted of groups of 15 vehicle-control animals of each sex from studies using the doses of 2 or 4 mg/kg, combined with

corresponding groups of 15 vehicle-control animals of each sex from the study using the dose of 1 mg/kg.

β-TGdR was toxic to rats at the doses used in this study. Mean body weights of the high- and low-dose rats of both sexes were lower than those of the corresponding vehicle controls throughout the study. There was also severe early mortality in the high-dose groups of both sexes and positive dose-related trends in mortality over the period of the bioassay. However, 66% of the low-dose males and 77% of the low-dose females survived until termination of the study.

In mice, β-TGdR was toxic at the doses originally selected. Mean body weights were not consistently affected; however, at the high dose only three males and seven females lived past week 7, and all were dead by week 25. In the mid-dose group, only 14% of the males and 6% of the females survived until termination of the study at week 79; in the low-dose group, the survival rate was 31% for the males and 29% for the females.

Because of the high mortality, time-adjusted statistical analyses were performed for both rats and mice.

In rats, the incidence of carcinomas of the ear canal (combined carcinomas and squamous-cell carcinomas) was statistically significant in both sexes. In males, the results of the test for dose-related trend were significant using either matched vehicle ($P = 0.046$) or pooled vehicle ($P = 0.014$) controls, but direct comparisons of dosed male rats with matched vehicle or pooled vehicle controls did not show significant differences (matched vehicle controls 0/10, pooled vehicle controls 0/28, low-dose 1/31, high-dose 2/7). In females, the results of the test for dose-related trend were significant using either matched vehicle ($P = 0.002$) or pooled vehicle ($P < 0.001$) controls, and the incidence in the high-dose group was significantly higher than that in either the matched vehicle ($P = 0.023$) or pooled vehicle ($P < 0.001$) controls (matched vehicle controls 0/9, pooled vehicle controls 0/28, low-dose 2/32, high-dose 6/13). There were no such ear canal tumors among 165 historical vehicle controls of either sex or among 220 female untreated controls at the laboratory, and only two such tumors occurred among 215 male untreated controls.

In mice, no tumors appeared in statistically significant incidences in the dosed groups compared with the matched vehicle controls, and there was no significant evidence of dose-related trend for any tumors. The incidences of the combination of lymphoma and leukemia were significantly higher in the matched vehicle controls of each sex than in the corresponding matched untreated controls (males: matched untreated controls 1/30, matched vehicle controls 19/29; females: matched untreated controls 2/30, matched vehicle controls 21/29). This high incidence in the matched vehicle controls may have been due to a systematic procedural problem associated with injection of the drug.

It is concluded that under the conditions of this bioassay, the low survival of the dosed and vehicle-control groups of mice, as well as the possible procedural problem that may have affected the incidences of tumors in these groups, does not allow a determination to be made of the carcinogenic potential of β-TGdR in this species. β-TGdR in the vehicle of 0.05% polysorbate 80 was, however, carcinogenic in rats, producing carcinomas of the ear canal in the females and possibly also in the males.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equiocal
Female Rats:	Positive
Male Mice:	Inadequate Study
Female Mice:	Inadequate Study

TR-58 Bioassay of Thio-TEPA for Possible Carcinogenicity (CAS No. 52-24-4)

Thio-TEPA is an ethyleneimine alkylating agent that was introduced in 1953 for clinical use in cancer chemotherapy. At one time thio-TEPA was an important therapeutic drug in the management of ovarian carcinoma. It has been used effectively in the treatment of Hodgkins disease, bronchogenic carcinoma, bladder cancer, retinoblastoma, and breast cancer and for the control of pleural, pericardial, and peritoneal neoplastic effusions.

A bioassay of thio-TEPA for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 31-39 rats of each sex were administered thio-TEPA in phosphate-buffered saline at one of three doses, either 0.7, 1.4, or 2.8 mg/kg body weight, three times per week for a maximum of 52 weeks, then observed for additional periods of time. The maximum time on study (administration of chemical and observation) was 86 weeks. The groups at the low dose were started 69 weeks after those at the mid and high doses, because of high mortalities observed in the groups at the higher doses. Matched controls consisted of groups of 10 untreated rats and 10 vehicle-control rats of each sex. Pooled-control groups also were used. Surviving control rats were killed at 82-87 weeks; surviving dosed rats were killed at 81 or 82 weeks.

Groups of 35 mice of each sex were administered thio-TEPA at one of two doses, either 1.15 or 2.3 mg/kg body weight, three times per week for a maximum of 52 weeks, then observed for a maximum additional period of 34 weeks. Matched controls consisted of groups of 15 untreated mice and 15 vehicle-control mice of each sex. Pooled controls also were used. Surviving control and dosed mice were killed at 86 or 87 weeks.

Thio-TEPA was toxic to both rats and mice, causing decreased mean body weight gains and early deaths in the mid- and high-dose rats and in the high-dose mice. Because of the early deaths, statistical analyses were based only on time-adjusted incidences of tumors. Since all high-dose male and female rats had died by 21 weeks, microscopic evaluation of tissues was performed only on the low- and mid-dose animals.

In rats, the incidence of combined neoplasms of the hematopoietic system (lymphoma, lymphocytic leukemia, or granulocytic leukemia) was significant in the males in both the low-dose ($P = 0.020$) and mid-dose ($P = 0.001$) groups, using pooled controls (pooled controls 0/29, low-dose 6/34; pooled controls 0/30, mid-dose 6/16).

Squamous-cell carcinoma of the skin or ear canal occurred at a significant incidence in the male rats in both the low-dose ($P = 0.009$) and mid-dose ($P = 0.023$) groups, using pooled controls (pooled controls 0/29, low-dose 7/33; pooled controls 0/30, mid-dose 3/13) and in the mid-dose females ($P < 0.001$), using pooled controls (pooled controls 0/28, mid-dose 8/21); in addition, two low-dose females had such tumors, with none occurring in the corresponding low-dose controls.

The incidence of adenocarcinoma of the uterus was significant in the mid-dose female rats ($P = 0.001$), using pooled controls (pooled controls 0/28, mid-dose 7/21); in addition, two low-dose females had adenocarcinoma of the uterus, with no such tumor occurring in the corresponding low-dose controls.

In rats, neuroepitheliomas (neuroblastomas) or nasal carcinomas occurred in three low-dose males, two low-dose females, and two mid-dose females. Although these are not statistically significant incidences, these tumors did not occur among control animals and no such tumors have occurred in 380 Sprague-Dawley control rats of each sex in other bioassays at the same laboratory. Thus, they may be associated with administration of the chemical.

In the high-dose groups of both male and female mice, but not in the low-dose groups, the incidences of lymphoma or lymphocytic leukemia were significantly higher ($P < 0.001$) for each sex than those of either the vehicle or pooled controls (males: vehicle controls 1/8, pooled controls 1/18, low-dose 2/24, high-dose 26/28; females: vehicle controls 0/14, pooled controls 0/29, low-dose 5/26, high-dose 32/32).

In the low-dose male mice squamous-cell carcinoma was found in the skin of seven animals, in the preputial glands of six animals, and in the ear canal of two animals. A carcinoma of the preputial gland was also found in a high-dose male. When the incidences of the tumors at the different sites were combined, the incidence in the low-dose group was statistically significant using either the vehicle ($P = 0.004$) or the pooled ($P < 0.001$) controls (vehicle controls 0/8, pooled controls 0/18, low-dose 14/24, high-dose 1/2).

It is concluded that under the conditions of this bioassay, thio-TEPA was carcinogenic in both Sprague-Dawley rats and B6C3F$_1$ mice. In the rats, the chemical induced squamous-cell carcinoma of the skin or ear canal in both males and females, and hematopoietic neoplasms in the males; in the mice, it induced lymphoma or lymphocytic leukemia in both sexes and squamous-cell carcinoma in the skin and associated glands of males.

Synonym: tris(1-aziridinyl)phosphine sulfide

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-59 Bioassay of Estradiol Mustard for Possible Carcinogenicity (CAS No. 22966-79-6)

A bioassay of the experimental anticancer drug estradiol mustard for possible carcinogenicity was conducted by administering the chemical by gavage to Sprague-Dawley rats and B6C3F$_1$ mice.

Groups of 35 rats and 34-36 mice of each sex were administered estradiol mustard at one of the following doses, either 0.62 or 1.25 mg/kg body weight for rats and either 15 or 30 mg/kg body weight for mice. The vehicle used for the test chemical consisted of 0.05% polysorbate 80 in phosphate-buffered saline. The rats and mice were dosed three times per week for 52 weeks, then observed for an additional 30-34 weeks. Controls consisted of groups of 10 rats and 15 mice of each sex that were not administered the chemical (untreated controls) and also of groups of 10 rats of each sex, 14 male mice, and 16 female mice administered the vehicle alone (vehicle controls). Pooled controls were also used. All surviving rats were killed at 84-86 weeks and all surviving mice at 82-86 weeks.

Mean body weights of male rats and male and female mice administered estradiol mustard were lower throughout the greater part of the study than those of corresponding vehicle or untreated controls; mean body weights of dosed female rats were unaffected. Administration of the test chemical had no significant effect on the survival of either male or female rats. A large number of dosed mice died prior to the end of the study. The numbers of dosed male mice which were at risk as long as 52 weeks were sufficient, however, for development of tumors appearing up to that time. Time-adjusted analysis and life-table analyses were applied to data obtained with the mice.

In rats, no tumors were observed in a statistically significant incidence in the animals administered estradiol mustard.

In mice, lymphoma or lymphocytic leukemia occurred at significant incidences in low-dose ($P = 0.018$) and high-dose ($P < 0.001$) groups of males compared with those in the pooled vehicle controls (controls 0/28, low-dose 6/32, high-dose 17/29) and at significant incidences in low-dose ($P = 0.020$) and high-dose ($P = 0.002$) groups of females compared with those in the corresponding vehicle controls (controls 0/14, low-dose 9/30, high-dose 11/23). In addition, the incidences of lymphoma were statistically significant for dose-related trend for both the males ($P < 0.001$) and the females ($P = 0.003$). Since lymphoma was observed in male mice as early as 25 weeks, life-table analyses of the incidence in each sex were performed.

The results indicated a dose association (P = 0.001) between the administration of estradiol mustard and the time of observation of lymphoma in either sex of mice.

In mice, alveolar/bronchiolar adenoma or carcinoma occurred at a significant incidence (P = 0.004) in the low-dose group of males compared with the pooled vehicle controls (controls 2/28, low-dose 12/30, high-dose 5/24) and at a significant incidence (P = 0.022) in the low-dose group of females compared with the pooled vehicle controls (controls 1/28, low-dose 7/27, high-dose 1/18). Sarcoma of the myocardium similarly occurred at a significant incidence (P = 0.015) in the low-dose group of males compared with the pooled vehicle controls (controls 0/28, low-dose 6/30, high-dose 2/24) and at a significant incidence (P = 0.002) in the low-dose group of females compared with the pooled vehicle controls (controls 0/28, low-dose 8/27, high-dose 1/12). The survival of both high-dose males and high-dose females was slightly lower than that of the respective low-dose groups and may account for the higher numbers of pulmonary tumors and myocardial sarcomas among low-dose mice of both sexes. The association of myocardial sarcoma with administration of the chemical in both dosed groups of each sex is strengthened by the fact that these tumors of the myocardium have not occurred in the more than 500 male and 500 female historical-control mice of this strain at the laboratory.

Squamous cell carcinoma of the stomach occurred in the dosed male mice (high-dose 2/29) and in the dosed female mice (low-dose 2/26, high-dose 2/14) but was absent in all controls. Although the incidences in this bioassay were too low to be statistically significant, the fact that no squamous-cell carcinomas of the stomach have occurred in the more than 500 male and 500 female historical-control mice of this strain at this laboratory indicates that these gastric tumors were related to the administration of the estradiol mustard.

It is concluded that under the conditions of this bioassay, estradiol mustard administered in a buffered saline vehicle was not carcinogenic in Sprague-Dawley rats. Estradiol mustard was carcinogenic in both male and female $B6C3F_1$ mice, inducing lymphoma, sarcoma of the myocardium, alveolar adenoma or carcinoma, and squamous-cell carcinoma of the stomach.

Synonym: estradiol, bis((p-bis(2-chloroethyl)-amino) phenyl)acetate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-60 Bioassay of Phenesterin for Possible Carcinogenicity (CAS No. 3546-10-9)

Phenesterin, an experimental anticancer agent, is a steroidal alkylating agent composed of the carboxylic acid ester of cholesterol and an aryl nitrogen mustard.

A bioassay of phenesterin for possible carcinogenicity was conducted by administering the chemical by gavage to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered phenesterin at one of two doses, either 5 or 10 mg/kg body weight, three times per week for 52 weeks, then observed for an additional 32 or 33 weeks. The vehicle used was 0.05% polysorbate 80 in buffered saline. Controls consisted of groups of 10 rats of each sex which received the vehicle (vehicle control) and 10 rats of each sex which were untreated (untreated control). All surviving rats were killed at 84 or 85 weeks.

Groups of 35 mice of each sex were administered the chemical at one of two doses, either 15 or 30 mg/kg body weight, three times per week for 52 weeks. The males receiving 15 mg/kg were observed for an additional period of 29 weeks, and those surviving to this time were then killed; the animals of the remaining groups were observed for additional periods of only 10-22 weeks, due to early deaths. Seventy-seven weeks after the foregoing groups were started, additional groups of 40 mice of each sex were started and were administered the chemical at 7 mg/kg body weight three times per week; administration of the chemical terminated at week 102 for the males and at week 88 for the females, due to deaths of all females at this time. Controls for the low-dose (7 mg/kg) groups of mice consisted of groups of 20 mice of each sex which received the vehicle (vehicle control) and 20 mice of each sex which were untreated (untreated control); controls for the mid-dose (15 mg/kg) and the high-dose (30 mg/kg) controls consisted of groups of 15 mice of each sex similarly receiving the vehicle or untreated. All surviving low-dose controls were killed at 104 weeks, and all surviving mid- and high-dose controls were killed at 81-84 weeks.

Phenesterin was toxic to rats and mice at the doses used, as shown by reduced mean body weights and survival. Time-adjusted analyses were used for evaluation of incidences of tumors in the female mice.

In female rats, a dose-related trend (P = 0.019) was present in adenocarcinoma of the mammary gland, using the pooled controls, and the incidences of the tumor in the individual dosed groups were significant (P < 0.009) when compared with those in the pooled controls (controls 1/18, low-dose 12/29, high-dose 12/30).

In male mice, the incidence of alveolar/bronchiolar carcinomas or combined alveolar/bronchiolar adenomas and carcinomas in the low-dose group (18/40) was significantly higher (P < 0.020) than that in the low-dose vehicle-control group (0/16). In female mice, seven low-dose animals had alveolar/bronchiolar adenomas and eight other low-dose animals had alveolar/bronchiolar car-

cinomas. When these tumors were combined, their time-adjusted incidence was significant (P = 0.004) when compared with that in the low-dose vehicle controls (controls 1/18, low-dose 15/35). The lower and nonsignificant incidences of these tumors observed in the mid- and high-dose groups may be due to the earlier mortality in these groups compared with the low-dose groups.

In each sex of mid- and high-dose mice, incidences of lymphoma and leukemia were dose related (P<0.005), using vehicle controls; they were also significant (P<0.018) in direct comparisons of mid- and high-dose groups of both sexes with respective vehicle controls (males: controls 0/14, mid-dose 9/29, high-dose 11/25; females, time-adjusted: controls 0/15, mid-dose 14/18, high-dose 17/19). The significance of the incidence of lymphoma and leukemia in the mid- and high-dose groups of males was increased (P<0.001) when the pooled-control group was used, both in the test for dose-related trend and in tests for direct comparisons of dosed groups with the controls.

In each sex of mice, sarcomas of the myocardium were found in all groups of dosed animals, but in no control animals (males: low-dose 5/40, mid-dose 7/29, high-dose 2/25; females: low-dose 8/34, mid-dose 2/7, high-dose 3/7). In males, the incidence in the mid-dose group was significant when compared with that in the pooled controls (P = 0.006); in females, the incidences in the low- and high-dose groups were significant (P<0.023).

It is concluded that under the conditions of this bioassay, phenesterin was carcinogenic in female Sprague-Dawley rats, producing adenocarcinomas of the mammary gland, and in both sexes of $B6C3F_1$ mice, producing alveolar/bronchiolar carcinomas, hematopoietic tumors, and myocardial sarcomas.

Synonym: cholesteryl p-bis(2-chloroethyl)-minophenylacetate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-61 Bioassay of Pentachloronitrobenzene for Possible Carcinogenicity (CAS No. 82-68-8)

Pentachloronitrobenzene (PCNB), a halogenated benzene derivative and agricultural pesticide, was selected for bioassay by the National Cancer Institute following its classification as a tumorigenic agent by the Secretary's Commission on Pesticides and Their Relationship to Environmental Health.

A bioassay of technical-grade pentachloronitrobenzene (PCNB) for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. PCNB was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The time-weighted average dietary concentrations of PCNB were, respectively, 10,064 and 5,417 ppm for male rats, 14,635 and 7,875 ppm for female rats, 5,213 and 2,606 ppm for male mice, and 8,187 and 4,093 ppm for female mice. After a 78-week period of compound administration, observation of the rats continued for an additional 33 to 35 weeks and observation of the mice continued for 14 or 15 additional weeks.

For each species, 20 animals of each sex were placed on test as controls and fed only the basal diet.

No rare or unusual tumors were observed during the histopathologic examinations and no statistically significant positive associations were demonstrated between chemical administration and the incidence of neoplasms in either sex of either species.

It is concluded that under the conditions of this bioassay PCNB was not carcinogenic in either Osborne-Mendel rats or $B6C3F_1$ mice.

Synonyms: quintozene; terrachlor; PCNB

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

Note: Pentachloronitrobenzene was subsequently studied by administration in feed to $B6C3F_1$ mice (See TR-325, reported 1978).

TR-62 Bioassay of Endosulfan for Possible Carcinogenicity (CAS No. 115-29-7)

Endosulfan is a synthetic chlorinated cyclodiene and was introduced in 1956 as a broad spectrum insecticide.

A bioassay of technical-grade endosulfan for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Endosulfan was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species.

The time-weighted average high and low dietary concentrations of endosulfan were, respectively, 952 and 408 ppm for the male rats, and 445 and 223 ppm for the female rats. In mice the high and low time-weighted average concentrations were, respectively, 6.9 and 3.5 ppm for the males and 3.9 and 2.0 ppm for the females. Twenty animals of each sex and species were placed on test as controls. The bioassay of high dose male rats was terminated during week 82, and the bioassay of low dose male rats was terminated during week 74. After a 78-week period of chemical administration, observation of female rats continued for 33 additional weeks and observation of mice continued for 14 additional weeks.

At the doses administered to rats in this study endosulfan was toxic, inducing a high incidence of toxic nephropathy in both sexes and testicular atrophy in males.

In both species high early mortality was observed in the male groups and no conclusions concerning the carcinogenicity of endosulfan can be drawn from this part of the bioassay. However, survival among females of both species was sufficient for meaningful statistical evaluation of the incidence of late-developing tumors. It is concluded that under the conditions of this bioassay, technical-grade endosulfan was not carcinogenic in female Osborne-Mendel rats or in female $B6C3F_1$ mice.

Synonym: 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Negative
Male Mice:	Inadequate Study
Female Mice:	Negative

TR-63 Bioassay of 4-Chloro-o-phenylenediamine for Possible Carcinogenicity (CAS No. 95-83-0)

4-Chloro-o-phenylenediamine, an aromatic amine used as an intermediate in dye production, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer reported among dye manufacturing industry workers.

A bioassay for the possible carcinogenicity of technical-grade 4-chloro-o-phenylenediamine was conducted using Fischer 344 rats and $B6C3F_1$ mice. 4-Chloro-o-phenylenediamine was administered in the feed, at either of two concentrations, to groups of 49 or 50 male and 50 female animals of each species. For male and female rats, the high and low time-weighted average dietary concentrations of 4-chloro-o-phenylenediamine were 1.0 and 0.5 percent, respectively. For male and female mice, the high and low time-weighted average dietary concentrations were 1.4 and 0.7 percent, respectively. After a 78-week period of chemical administration, observation of the rats continued for up to an additional 28 weeks and observation of the mice continued for up to an additional 18 weeks. Fifty animals of each species and sex were placed on test as controls for the chronic bioassay.

There was a statistically significant positive association between increased dosage and accelerated mortality in female rats and male mice; however, survival among all groups of was adequate for meaningful statistical analysis of late-developing tumors.

In male and female rats receiving the test chemical, a significantly increased incidence of neoplasms of the urinary bladder occurred. Neoplastic nodules in the liver and tumors of the forestomach may also have been related to administration of the chemical. A significantly increased incidence of hepatocellular carcinomas occurred in chemically treated male and female mice.

It is concluded that under the conditions of this bioassay 4-chloro-o-phenylenediamine was carcinogenic in Fischer 344 rats and $B6C3F_1$ mice, including tumors of the urinary bladder and forestomach in both sexes of rats and hepatocellular carcinomas in both sexes of mice.

Synonyms: 4-chloro-1,2-benzenediamine; 4-chloro-1,2-diaminobenzene; Ursol Olive 6G

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-64 Bioassay of 1-Nitronaphthalene for Possible Carcinogenicity (CAS No. 86-57-7)

1-Nitronaphthalene is used as an intermediate for the preparation of 1-naphthylamine, which is used in the manufacture of numerous dyes and intermediates, and in the production of rodenticides. 1-Nitronaphthalene is also sulfonated to produce 1-nitronaphthalene-5-sulfonic acid, a dye intermediate. 1,5- and 1,8-Dinitronaphthalenes, produced by further nitration of 1-nitronaphthalene, have had limited use in the dye industry. 1-Nitronaphthalene is also used as a deblooming agent for petroleum and oils (in concentrations of 2-3 parts/1,000 parts oil), and as a modifier to decrease the burning rate of explosives.

A bioassay of technical-grade 1-nitronaphthalene for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. 1-Nitronaphthalene was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low time-weighted average concentrations used in the chronic study were, respectively, 0.18 and 0.06 percent for rats and 0.12 and 0.06 percent for mice. After a 78-week period of chemical administration, the rats were observed for an additional period of up to 31 weeks and the mice for an additional period of up to 20 weeks. For rats 50 animals of each sex were placed on test as controls for the low dose groups and 25 of each sex for the high dose groups. For mice 50 animals of each sex were placed on test as controls for each dosed group.

In both species adequate numbers of animals in all groups survived sufficiently long for the development of late-appearing tumors; however, no compound-related increase in the incidence of neoplasms, nonneoplastic lesions, or other toxic effects was evident.

Under the conditions of this bioassay 1-nitronaphthalene was not demonstrated to be carcinogenic in Fischer 344 rats or $B6C3F_1$ mice.

Synonyms: alpha-nitronaphthalene, nitronaphthalene

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-65 Bioassay of Chloropicrin for Possible Carcinogenicity (CAS No. 76-06-2)

Chloropicrin is an agricultural fumigant, once widely used but now being phased out. It was developed as a tear gas, but was found to be useful as a fumigant in 1918. The primary use of chloropicrin as a fumigant was in the treatment of stored grain. It also functions as a nematicide, fungicide, and insecticide when used as a soil fumigant prior to planting.

A bioassay of technical-grade chloropicrin for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. Chloropicrin in corn oil was administered 5 days a week by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. Time-weighted average dosages of 25 mg/kg/day for low dose male rats and 20 mg/kg/day for low dose female rats were administered during weeks 1 through 33, then administered cyclically (1 dose-free week followed by 4 weeks of administration) from weeks 34 through 78. Time-weighted average dosages of 26 mg/kg/day for high dose male rats and 22 mg/kg/day for high dose female rats were administered from weeks 1 through 17, weeks 31 through 33, and cyclically (1 dose-free week followed by 4 weeks of administration) during weeks 34 through 78. Time-weighted average dosages of 66 and 33 mg/kg/day, respectively, for male and female mice were administered for 78 weeks. These dosing regimens were followed by observation periods of 32 weeks for rats and 13 weeks for mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not gavaged.

A high incidence of early death was observed among chloropicrin-dosed rats. Deaths among dosed rats occurred as early as week 1 for females and week 6 for males. Median survival was week 48 for high dose males, week 54 for low dose males, week 70 for high dose females and week 59 for low dose females. Statistical tests indicate a positive association between chloropicrin dosage and mortality of rats.

No neoplasms were observed at higher incidences in dosed than control rats. In rats of both sexes, incidences of adenoma of the pituitary and of adenocarcinoma or fibroadenoma of the mammary gland were higher in control groups than dosed groups. It is likely that most dosed rats did not survive long enough to be at risk from late-appearing tumors.

A rapid decrease in survival after the first year of the study was observed among high dose mice of both sexes. Survival of high dose male mice decreased from 80 percent in week 54 to 26 percent in week 90. Survival of high dose female mice decreased from 82 percent in week 54 to 36 percent in week 90. Statistical tests indicated a positive association between chloropicrin dosage and mortality of mice.

In chloropicrin-dosed mice, proliferative lesions of the squamous epithelium of the forestomach included two carcinomas and a papilloma. Although these tumors were uncommon in control animals, statistical analysis did not demonstrate that they were related to administration of chloropicrin. Other proliferative lesions of the forestomach occurring at an increased incidence in dosed mice were acanthosis and hyperkeratosis. No statistically significant increase of tumor incidence was observed in mice.

The bioassay of chloropicrin using Osborne-Mendel rats did not permit an evaluation of carcinogenicity because of the short survival time of dosed animals. The bioassay of chloropicrin using B6C3F$_1$ mice did not provide conclusive statistical evidence for the carcinogenicity of this compound.

Synonyms: trichloronitromethane, nitrochloroform

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Negative
Female Mice:	Negative

TR-66 Bioassay of 1,1-Dichloroethane for Possible Carcinogenicity (CAS No. 75-34-3)

1,1-Dichloroethane is used as a chemical intermediate and as a solvent for extraction and degreasing.

A bioassay of technical-grade 1,1-dichloroethane for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. 1,1-Dichloroethane in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species, 5 days a week for a period of 78 weeks, followed by an observation period of 33 weeks for rats and 13 weeks for mice.

A preliminary subchronic toxicity test, consisting of 6 weeks of 1,1-dichloroethane administration at five dosage levels followed by 2 weeks of observation, was performed for the purpose of selecting initial dosages. Subsequent dosage adjustments were made during the course of the study. The high and low time-weighted average dosages of 1,1-dichloroethane were, respectively, 764 and 382 mg/kg/day for male rats; 950 and 475 mg/kg/day for female rats; 2,885 and 1,442 mg/kg/day for male mice; and 3,331 and 1,665 mg/kg/day for female mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil at the same times that dosed animals were gavaged with 1,1-dichloroethane mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

Survival was poor in all rat groups and several mouse groups. Survival at the end of the study in the untreated control, vehicle control, low dose, and high dose groups was, respectively, 30, 5, 4, and 8 percent in male rats; 40, 20, 16 and 18 percent in female rats; 35, 55, 62 and 32 percent in male mice; and 80, 80, 80 and 50 percent in female mice. Pneumonia was observed in 80 percent of the rats in this bioassay.

There were dose-related marginal increases in mammary adenocarcinomas and in hemangiosarcomas among female rats and there was a statistically significant increase in the incidence of endometrial stromal polyps among dosed female mice as compared to controls. These findings are indicative of the possible carcinogenic potential of the test compound. However, it must be recognized that under the conditions of this bioassay there was no conclusive evidence for the carcinogenicity of 1,1-dichloroethane in Osborne-Mendel rats or $B6C3F_1$ mice.

Synonym: ethylidene dichloride

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Equivocal
Male Mice:	Negative
Female Mice:	Equivocal

TR-67 Bioassay of a Mixture of Aspirin, Phenacetin, and Caffeine for Possible Carcinogenicity (CAS No. 8003-03-0)

APC, an abbreviation often used for mixtures of aspirin, phenacetin and caffeine, is a nonprescription analgesic preparation sold for relief of headache, muscular aches and pains, arthritis, and other common afflictions. APC is also antipyretic and anti-inflammatory and also acts as a central stimulant.

A bioassay of a mixture of aspirin, phenacetin, and caffeine (APC) for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. APC was administered in the feed, at either of two concentrations, to groups of 50 male and 49 or 50 female animals of each species. For each species, 50 animals of each sex were placed on test as controls. The high dose used in the chronic study for the male and female rats and mice was 1.4 percent. The low dose administered to the male and female rats and mice was 0.7 percent. After a 78-week period of compound administration, observation of the rats continued for up to an additional 35 weeks and observation of the mice continued for an additional 16 weeks.

No significant association was established between administration of APC and mortality in rats or female mice; however, there was a significant positive association between treatment and mortality in male mice. For both species the survival in all groups was adequate for statistical analysis of tumor incidence.

In rats, a variety of endocrine tumors were observed with greater frequency in the male rats treated with APC than in the male control rats. These same tumors were not observed with similar frequencies in the female rats or in the mice of either sex. The endocrine tumors observed most frequently in the treated male rats were adenomas and carcinomas of the pituitary gland. The incidences of these tumors proved to be statistically inconclusive.

In rats, a transitional-cell carcinoma of the bladder was observed in one low dose and one high dose females. A tubular-cell adenocarcinoma of the kidney was seen in one low dose female and one low dose male. A fifth neoplasm, a transitional-cell papilloma of the kidney, was seen in one high dose female. The occurrence of these urinary tumors, although considered important, was not statistically significant.

In mice, there was no statistically significant positive association between APC administration and the incidence of tumors in either sex.

Under the conditions of this bioassay evidence was not sufficient for the carcinogenicity of APC in Fischer 344 rats or in $B6C3F_1$ mice.

Synonyms for APC: 2-(acetyloxy)-benzoic acid, mixture with N-(4-ethoxy-phenzl) acetamide and 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione

Synonym for aspirin: acetylsalicylic acid

Synonym for phenacetin: p-acetophenetidide

Synonym for caffeine: theine, 1,3,7-trimethylxanthine

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Equivocal
Male Mice:	Negative
Female Mice:	Negative

TR-68 Bioassay of Hexachloroethane for Possible Carcinogenicity (CAS No. 67-72-1)

Hexachloroethane is used as a veterinary anthelmintic for control of liver and stomach flukes in domestic animals. It is also used as a solvent, a camphor substitute in the preparation of Celluloid®, a rubber vulcanizing accelerator, a retarding agent in fermentation, and in explosives, pyrotechnics, and smoke devices.

A bioassay for possible carcinogenicity of technical-grade hexachloroethane was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Hexachloroethane in corn

oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The chemical was administered 5 days a week, cyclically for 44 of 78 weeks in rats and continuously for 78 weeks in mice, followed by an observation period of 33 or 34 weeks for rats and 12 or 13 weeks for mice. The high and low time-weighted average dosages of hexachloroethane were, respectively, 423 and 212 mg/kg/day for male and female rats and 1179 and 590 mg/kg/day for male and female mice. For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with pure corn oil at the same rate as the high dose group of the same sex. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

A statistically significant association between increased dosage and accelerated mortality was observed in male and female rats but not in mice of either sex.

Toxic tubular nephropathy was observed in all groups of treated animals.

Statistical evaluation of the incidences of hepatocellular carcinomas revealed a significant positive association between hexachloroethane administration and tumor incidence in both male and female mice. No statistical significance was attributed to the incidence of any neoplasm in rats of either sex.

No evidence was provided for the carcinogenicity of the compound in Osborne-Mendel rats. It is concluded that under the conditions of this bioassay, hexachloroethane was carcinogenic in $B6C3F_1$ mice, inducing hepatocellular carcinomas in both sexes.

Synonyms: carbon hexachloride, perchloroethane, ethylene hexachloride

Trade Name: Avlothane®

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

Note: Hexachloroethane was subsequently studied by gavage administration to F344 rats (See TR-361, reported 1989).

TR-69 Bioassay of Azinphosmethyl for Possible Carcinogenicity (CAS No. 86-50-0)

Azinphosmethyl is a broad-spectrum, organophosphorus insecticide that was first produced in 1953 and is used solely for agricultural purposes. In 1974, 3.1 million pounds were estimated to be have been used in the United States on the following crops: alfalfa, cotton, deciduous fruits and nuts, tobacco, vegetables and some miscellaneous items.

A bioassay of technical-grade azinphosmethyl for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered azinphosmethyl at one of two doses for 80 weeks, then observed for 34 or 35 weeks. Time-weighted average doses of either 78 or 156 ppm were used for the males. Initial doses of 62.5 or 125 ppm used for the females were maintained throughout the bioassay. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched controls combined with 95 male and 95 female untreated rats from similar bioassays of 10 other test chemicals. All surviving rats were killed at 114 or 115 weeks.

Groups of 50 mice of each sex were administered azinphosmethyl at one of two doses for 80 weeks, then observed for 12 or 13 weeks. The doses were either 31.3 or 62.5 ppm for the males and either 62.5 or 125 ppm for the females. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched controls combined with 130 male and 120 female untreated mice from similar bioassays of 11 other test chemicals. All surviving mice were killed at 92 or 93 weeks.

High- and low-dose male rats and mice and high-dose female rats and mice had lower mean body weights than corresponding matched controls throughout the bioassay. Typical signs of organophosphate intoxication were observed in a few animals of both species, and included hyperactivity, tremors, and dyspnea. Sufficient numbers of animals were at risk in each species for development of late-appearing tumors.

A great many tumors of the endocrine organs were observed in both dosed male and dosed female rats. Those of the adrenal in dosed males and females, the follicular cells of the thyroid in dosed females, the anterior pituitary in dosed males, and the parathyroid in dosed males occurred at statistically significant incidences when compared with pooled controls, but not with matched controls, and they were not considered to be related to administration of the test compound. The incidences of tumors of the pancreatic islets and of the follicular cells of the thyroid in the male rats suggest, but do not clearly implicate, azinphosmethyl as a carcinogen in these animals.

In mice of each sex there were no increased incidences of tumors that could be related to administration of the test chemical.

It is concluded that under the conditions of this bioassay, neoplasms of the thyroid and pancreatic islets suggest but do not provide sufficient evidence for the carcinogenicity of azinphosmethyl in male Osborne-Mendel rats. Azinphosmethyl was not shown to be carcinogenic in female Osborne-Mendel rats or in $B6C3F_1$ mice of either sex.

Synonym: 0,0-dimethyl-S-[(4-oxo-1,2,3-benzotriazin-3-(4H)-yl)methyl]phosphorodithioate

Trade Name: Guthion®

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-70 Bioassay of Parathion for Possible Carcinogenicity (CAS No. 56-38-2)

Parathion is an organophosphorus pesticide that is relatively nonpersistent in the environment, with high activity against insects and mites. It is used as an insecticide and acaricide on a wide variety of fruit and nut trees, berries, vegetables, field crops, and ornamental plants.

A bioassay for possible carcinogenicity of technical-grade parathion was conducted by administering the test chemical in the diet to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered parathion at one of two doses for 80 weeks, then observed for 32 or 33 weeks. Time-weighted average doses for males were 32 or 63 ppm; for females, they were 23 or 45 ppm. All surviving rats were killed at 112 or 113 weeks. Groups of 50 mice of each sex were administered parathion at one of two doses, either 80 or 160 ppm. The low-dose males were administered parathion for 71 weeks; the high-dose males for 62 weeks; and the low- and high-dose females for 80 weeks. The animals were then maintained for observation and all surviving mice were killed at 89 or 90 weeks. Matched controls consisted of groups of 10 untreated rats or mice of each sex; pooled controls of rats or mice taken from similar bioassays of other test chemicals were also used.

Mean body weights of high-dose male and female rats and of high- and low-dose male mice were generally lower than those of the matched controls during the period of administration of the chemical. Mean body weights of the other groups of dosed rats and mice did not differ appreciably from those of the matched controls. Since body weights and survival of the female mice were not affected, female mice may have been able to tolerate a higher dose. Sufficient numbers of male and female animals of both species were at risk for the development of late-appearing tumors.

In both male and female rats, the incidences of cortical adenomas or carcinomas of the adrenal showed dose-related trends ($P<0.001$) using pooled controls and, in direct comparisons, were higher in the high-dose groups ($P<0.001$) than in the pooled controls (males: pooled controls 3/80, matched controls 0/9, low-dose 7/49, high-dose 11/46; females: pooled controls 4/78, matched controls 1/10, low-dose 6/47, high-dose 13/42). Most of the tumors were adenomas. When the matched controls were used, dose-related trends in incidences of the adrenal tumors were significant (males, $P=0.048$; females, $P=0.028$); in direct comparisons, however, the incidences of the tumors in the individual groups did not differ significantly from those in corresponding matched controls. The incidences of the tumors in the dosed male and female rats were higher than those in corresponding historical controls (males 8/148, females 5/180).

In mice, no tumors occurred in either sex at incidences that were significantly higher in the dosed groups than in the corresponding control groups.

It is concluded that under the conditions of this bioassay, parathion was not carcinogenic to $B6C3F_1$ mice. In the male and female Osborne-Mendel rats receiving parathion in their diet, there was a higher incidence of cortical tumors of the adrenal than in pooled or historical controls, suggesting that parathion is carcinogenic to this strain of rat.

Synonym: 0,0-diethyl-O-4-nitrophenylphosphorothioate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Negative
Female Mice:	Negative

TR-71 Bioassay of L-Tryptophan for Possible Carcinogenicity (CAS No. 73-22-3)

L-Tryptophan is an essential amino acid for humans, and a precursor of the neurohormones serotonin (5-hydroxytryptamine) and melatonin (N-acetyl-5-methoxytryptamine), and the B vitamin nicotinic acid. It is found in small concentrations in casein, and in many foods.

A bioassay of the amino acid L-tryptophan for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered L-tryptophan at one of two doses, either 25,000 or 50,000 ppm, 5 days per week for 78 weeks, and then observed for 26 or 27 weeks. Matched controls consisted of groups of 15 rats or 15 mice of each sex. All surviving rats and mice were killed at 104 or 105 weeks.

L-Tryptophan had little toxic effect on the rats; mean body weight loss was minimal and survival of dosed groups of both sexes was high. In the mice, mean body weights of dosed animals were lower than those of controls throughout most of the bioassay, particularly in the females. Sufficient numbers of rats were at risk to termination of the study for development of late-appearing tumors, and sufficient numbers of mice were at risk beyond 52 weeks of the study for development of tumors.

No neoplasms occurred in a statistically significant incidence among dosed rats when compared with controls.

In both male and female mice, neoplasms of the hematopoietic system occurred at higher incidences in the low-dose groups than in the matched-control groups (males: controls 0/12, low-dose 9/34, high-dose 2/33; females: controls 2/13, low-dose 6/33, high-dose 1/35). These incidences, however, are not statistically significant, using the Bonferroni correction, and therefore, no tumors are considered to be related to the administration of the test chemical.

It is concluded that under the conditions of this bioassay, L-tryptophan was not carcinogenic for Fischer 344 rats or $B6C3F_1$ mice.

Synonym : L-α-amino-β-indolepropionic acid

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-72 Bioassay of Phenoxybenzamine Hydrochloride for Possible Carcinogenicity (CAS No. 63-92-3)

Phenoxybenzamine hydrochloride is an antihypertensive agent that is used in controlling specific hypertensive crises such as those that result from high blood levels of sympathomimetic amines.

A bioassay of phenoxybenzamine hydrochloride for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered phenoxybenzamine hydrochloride at one of two doses, either 5 or 10 mg/kg body weight, three times per week for 52 weeks, then observed for an additional 31 or 32 weeks. The vehicle used for most of the period of the bioassay was 6% propylene glycol in saline, although other vehicles, including 0.05% polysorbate 80 in saline, were used at the beginning. Controls consisted of groups of 10 vehicle controls and 10 untreated controls of each sex. All surviving rats were killed at 83-85 weeks.

Groups of 35 mice of each sex were administered phenoxybenzamine hydrochloride at one of two doses, either 12.5 or 25 mg/kg body weight, three times per week for 50 or 52 weeks, then observed for an additional 31-33 weeks. Controls consisted of groups of 15 males and 15 females which were administered the vehicle (vehicle controls), and groups of 14 males and 16 females which were untreated (untreated controls). All surviving mice were killed at 83-85 weeks.

Mean body weights of the low-dose male rats, low- and high-dose female rats, and low-dose male and female mice were comparable to those of the untreated and vehicle controls. The mean body weights of the high-dose male rats and the high-dose male and female mice, which died early, were lower than those of the controls.

Sarcoma of the abdominal cavity (peritoneum) was found in dosed animals of both species, but did not occur in either the untreated or vehicle controls. In male rats, this lesion occurred with a significant dose-related trend ($P>0.001$), using a vehicle controls, and also at significant incidences in direct comparisons of the dosed groups with the vehicle controls (controls 0/10, low-dose 11/31, $P=0.027$; high-dose 16/20, $P<0.001$). In female rats, the lesion occurred at a significant incidence in the high-dose group compared with the vehicle controls (controls 0/9, high-dose 16/30, $P=0.004$). None were observed among low-dose females.

In the mice, sarcoma of the abdominal cavity (peritoneum) occurred at a high and statistically significant incidence in the high-dose groups of each sex compared with vehicle controls (males: controls 0/15, high-dose 17/21, $P<0.001$; females: controls 0/13, high-dose 16/20, $P<0.001$). None were observed among low-dose groups. The morphology of the sarcoma was similar in the rats and the mice.

It is concluded that under the conditions of this bioassay, phenoxybenzamine hydrochloride was carcinogenic (sarcomagenic) for the peritoneum of both sexes of Sprague-Dawley rats and $B6C3F_1$ mice.

Synonym: N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-73 Bioassay of Allyl Chloride for Possible Carcinogenicity (CAS No. 107-05-1)

Allyl chloride is an extremely useful chemical intermediate since it can react both as an organic halide and as an olefin. Most derivatives of allyl chloride do not reach on end-use market themselves, but are part of further syntheses. Important "first generation" derivatives of allyl chloride include glycerol, epichlorohydrin, and allyl alcohol. Other derivatives include medicinals, such as barbiturates, diuretics, and herbicides.

A bioassay for possible carcinogenicity of technical-grade allyl chloride was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. At initiation of the study the rats were approximately 6 weeks old and the mice approximately 5 weeks old. Allyl chloride in corn oil was administered by gavage to two groups of each species for 5 days a week for 78 weeks, followed by observation periods of 30 to 33 weeks for the rats and 14 weeks for the mice. The time-weighted average dosages were, respectively, 77 and 57 mg/kg/day for high and low dose male rats; 73 and 55 mg/kg/day for high and low dose female rats; 199 and 172 mg/kg/day for high and low dose male mice; and 258 and 129 mg/kg/day for high and low dose female mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were intubated with corn oil at the same time that dosed animals were gavaged with allyl chloride in corn oil. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

Survival of high dose male mice and high dose rats of both sexes was extremely poor. Fifty percent of the high dose male mice were dead by week 27; the 10 members of this group that survived past week 48 were sacrificed in week 56. Among the high dose rats, 50 percent of the males had died by week 14 and 50 percent of the females had died by week 38. Because of early mortality in these groups, the number of animals surviving long enough to be at risk from late-developing tumors was not adequate for meaningful statistical analysis.

In this bioassay, squamous-cell carcinomas of the forestomach in male and female mice and squamous-cell papillomas of the forestomach in female mice occurred in incidences that were higher than in historical controls. No other neoplasms occurred in statistically significant increased incidences in dosed rats or mice

Under the conditions Of this bioassay no convincing evidence was presented for the carcinogenicity of allyl chloride in Osborne-Mendel rats of either sex. The results are suggestive that allyl chloride is carcinogenic in male and female $B6C3F_1$ mice since the compound, when administered by gavage caused a low incidence of neoplastic and nonneoplastic lesions of the forestomach.

Synonyms: 3-chloro-1-propene; 3-chloropropene; chloropropylene

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Equivocal

TR-74 Bioassay of 1,1,2-Trichloroethane for Possible Carcinogenicity (CAS No. 79-00-5)

1,1,2-Trichloroethane, an aliphatic chlorinated hydrocarbon, is used as a chemical intermediate in the production of vinylidene chloride. Other applications include use in adhesives, in the production of teflon tubing, in lacquer, and in coating formulations, and as a solvent for fats, oil, waxes, and other products.

A bioassay of technical-grade 1,1,2-trichloroethane for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. 1,1,2-Trichloroethane in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species, 5 days a week for a period of 78 weeks, followed by an observation period of up to 35 weeks for rats and up to 13 weeks for mice.

The high and low time-weighted average dosages of 1,1,2-trichloroethane were, respectively, 92 and 46 mg/kg/day for male and female rats, and 390 and 195 mg/kg/day for the male and female mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil at the same rate as the high dose group of the same sex. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

No neoplasms were observed at statistically significant incidences in male or female rats.

In both male and female mice, administration of 1,1,2-trichloroethane was associated with a significantly increased incidence of hepatocellular carcinomas. Hepatocellular carcinomas were observed in 2/17 (12 percent) untreated control males, 2/20 (10 percent) vehicle control males, 18/49 (37 percent) low dose males, and 37/49 (76 percent) high dose males. Hepatocellular carcinomas were also observed in 2/20 (10 percent) untreated control females, 0/20 vehicle control females, 16/48 (33 percent) low dose females, and 40/45 (89 percent) high dose females. Both the Fisher exact test comparing tumor incidences of dosed to control groups and the Cochran-Armitage test for positive dose-related trend indicated a highly significant ($P<0.001$) association between hepatocellular carcinomas in all mouse groups and the administration of 1,1,2-trichloroethane.

A positive dose-related association between administration of 1,1,2-trichloroethane and the incidence of pheochromocytoma of the adrenal gland was indicated by the Cochran-Armitage test for mice of both sexes. Fisher exact tests confirmed these results for high dose female mice but not for other mouse groups. There were no other neoplasms for which statistical tests indicated a positive association between dosage and tumor incidence in mice.

The results of this study do not provide convincing evidence for the carcinogenicity of 1,1,2-trichloroethane in Osborne-Mendel rats.

Under the conditions of this bioassay 1,1,2-trichloroethane is carcinogenic in $B6C3F_1$ mice, causing hepatocellular carcinomas and adrenal pheochromocytomas.

Synonyms: vinyltrichloride; beta-trichloroethane

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-75 Bioassay of Chlorobenzilate for Possible Carcinogenicity (CAS No. 510-15-6)

Chlorobenzilate is an agricultural pesticide used for mite control on citrus crops. Additionally, it is effective against mites in orchards, vineyards, tea plantations, field crops, and ornamental plants. Since bees are not severely affected by chlorobenzilate, it is also used to control the tracheal mite of this insect.

A bioassay of technical-grade chlorobenzilate for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Chlorobenzilate was

administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Chlorobenzilate was administered for 78 weeks followed by an observation period of 12 or 13 additional weeks in mice and 32 or 33 additional weeks in rats.

The time-weighted average dietary concentrations of chlorobenzilate were 2,995 and 1,600 ppm for high and low dose male rats, respectively, and 2,229 and 1,175 ppm for high and low dose female rats. Mice received time-weighted average high and low dietary concentrations of 7,846 and 4,231 ppm, respectively, for males and 5,908 and 3,200 ppm, respectively, for females.

Survival in both species was high (over 68 percent of the high dose rats and over 82 percent of the high dose mice survived on test until the end of the study). Dose-related mean body weight depression, observed in both species, indicated that the maximum dose for optimal bioassay sensitivity was used in the high dose groups.

An increased incidence of hepatocellular carcinomas was observed in dosed mice, i.e., 4/19 (21 percent) in control males, 32/48 (67 percent) in low dose males, 22/45 (49 percent) in high dose males, 0/20 in control females, 11/49 (22 percent) in low dose females, and 13/50 (26 percent) in high dose females.

There was a statistically significant positive association between the administration of chlorobenzilate and the appearance of cortical adenoma of the adrenal gland in low dose male and high dose female rats. Although suggestive, the findings of a low incidence of benign adrenal tumors was not considered sufficient evidence to establish the carcinogenicity of chlorobenzilate for the Osborne-Mendel rat.

Under the conditions of this bioassay, orally administered chlorobenzilate was carcinogenic in male and female $B6C3F_1$ mice, causing an increased incidence of hepatocellular carcinomas. The results do not, however, provide sufficient evidence for the carcinogenicity of chlorobenzilate in Osborne-Mendel rats.

Synonyms: 4,4'-dichlorobenzilic acid, ethyl ester; ethyl 4,4'-dichlorobenzilate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Positive
Female Mice:	Positive

TR-76 Bioassay of Tris (2,3-Dibromopropyl) Phosphate for Possible Carcinogenicity (CAS No. 126-72-7)

Tris (2,3-dibromopropyl) phosphate (TBP) is a compound that has been widely used as a flame retardant for synthetic fabrics, particularly those made into sleepwear for infants and young children. In addition to its use by the textile industry, TBP is also a fire retardant additive for polystyrene and polyurethane foams, polyvinyl chloride and phenolic resins, intumescent and nonintumescent paints, paper coatings, and rubber.

A bioassay of technical-grade tris (2,3-dibromopropyl) phosphate (TBP) for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. TBP was administered in the feed, at either of two concentrations, to groups of 55 male and 55 female rats, and 50 male and 50 female mice. The high and low dietary concentrations of TBP administered were, respectively, 100 and 50 ppm for the male and female rats, and 1,000 and 500 ppm for the male and female mice. After a 103-week dosing period, observation of the rats and mice continued for 1 or 2 additional weeks. For each species, 55 animals of each sex were placed on test as controls. No TBP was added to their diet.

In both species, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

Kidney tubular-cell adenomas were observed at incidences which were significant for dosed rats of both sexes by all statistical tests applied. For male rats there was a significant positive association between the incidence of kidney tubular-cell adenocarcinomas and dietary concentration of TBP. Other neoplastic lesions appearing in the treated rats were not statistically significant when compared with the control groups.

Among mice, a number of malignant and benign tumors were associated with TBP administration. These tumors included renal tubular-cell carcinoma and adenoma; squamous-cell papilloma and carcinoma of the forestomach; hepatocellular carcinoma and adenoma; and bronchiolar/alveolar adenoma and carcinoma.

Renal tubular-cell carcinomas were observed at a statistically significant incidence in male mice but none were observed in females. Tubular-cell adenomas were observed in treated mice of both sexes, but not in their respective controls. The incidence of tubular-cell adenomas was significant in male mice but not in females.

Squamous-cell carcinomas were observed in forestomachs of mice of both sexes but not in their respective controls. The incidence was significant in females but not in males. The incidences of squamous-cell papillomas of the forestomach were significant in mice of both sexes.

Incidences of hepatocellular carcinoma and hepatocellular adenoma were each significant in female mice. Tumor incidence among male mice was not significant for hepatocellular carcinomas or hepatocellular adenomas.

The proportion of mice of each sex having bronchiolar/alveolar adenoma or carcinoma or both had a significant positive dose-related trend. The incidence of bronchiolar/alveolar carcinomas exhibited a significant positive dose-related trend for males, but not for females.

It is concluded that under the conditions of this study orally administered TBP was carcinogenic to $B6C3F_1$ mice, causing increased incidences of tumors in livers, lungs, and stomachs of female mice and in kidneys, lungs, and stomachs of male mice. TBP was also carcinogenic in Fischer 344 rats, causing an increased incidence of kidney tumors in both sexes.

Synonyms: 2,3-dibromo-1-propanol phosphate (3:1); tris (dibromopropyl) phosphate; Firemaster T23P; Tris; TBP

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-77 Bioassay of Pyrimethamine for Possible Carcinogenicity (CAS No. 58-14-0)

A bioassay of pyrimethamine, a prophylactic antimalarial, for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered pyrimethamine 5 days per week at one of two doses, either 200 or 400 ppm for the rats and either 500 or 1,000 ppm for the mice. The animals were administered the chemical for 78 weeks, then observed for 26 or 27 additional weeks. Matched controls consisted of 15 untreated rats and 15 untreated mice of each sex; pooled controls consisted of the matched controls combined with 30 untreated rats and 30 untreated mice from similar bioassays of two other test compounds. All surviving rats and mice were killed at 102-105 weeks.

Mean body weights of the rats and mice fed diets containing pyrimethamine were slightly lower than those of the matched controls. Survival of the rats was not affected adversely by the chemical. In mice, survival rates of both dosed and matched-control males were low, with nearly two-thirds of the dosed and one-half of the control mice dying by week 52. Some of the deaths were associated with respiratory infections and may not have been related to administration of the chemical. Numbers of animals at risk in the dosed and control groups of female mice were adequate, however, for the development of late-appearing tumors.

In rats of each sex, no neoplastic lesions were found at a statistically significant incidence in the groups fed the pyrimethamine as compared with control groups. An increased frequency of bone-marrow atrophy occurred in both male and female dosed groups.

In male mice, the markedly decreased life spans may have prevented the observation of late-appearing tumors, since only two tumors were observed, one in a high-dose mouse and one in a low-dose mouse. In female mice, no neoplastic lesions were found at a statistically significant incidence in the groups fed the pyrimethamine as compared with control groups.

It is concluded that under the conditions of this bioassay, pyrimethamine was not carcinogenic for male or female Fischer 344 rats or for female $B6C3F_1$ mice. The carcinogenic potential of pyrimethamine for male $B6C3F_1$ mice cannot be assessed by this bioassay, because of the markedly reduced life span.

Synonym: 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Inadequate Study
Female Mice:	Negative

TR-78 Bioassay of ICRF-159 for Possible Carcinogenicity (CAS No. 21416-87-5)

A bioassay of the experimental anticancer drug ICRF-159 for possible carcinogenicity was conducted by administering the compound by intraperitoneal injection to Sprague-Dawley rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice were injected three times per week with ICRF-159 in buffered saline at one of the following doses, either 48 or 96 mg/kg body weight for the rats and either 40 or 80 mg/kg body weight for the mice. Both rats and mice were dosed for 52 weeks, then observed for 29-34 additional weeks. Untreated-control and vehicle-control groups each consisted of 10 rats and 15 mice of each sex; pooled-control groups consisted of the 10 vehicle controls of each sex of the rats combined with 30 vehicle controls of each sex of rats from similar bioassays of three other chemicals and the 15 vehicle controls of each sex of the mice combined with 30 vehicle controls of each sex of mice from similar bioassays of two other chemicals. All surviving rats were killed at 81-86 weeks; all surviving mice, at 86 weeks.

Mean body weights were depressed in rats and mice administered ICRF-159, and mortality was dose related among male and female rats and male mice. The high mortality among the male rats may have been associated with inflammatory lesions observed in the lungs, the liver, and the pleural and peritoneal cavities. Sufficient numbers of female rats and of both male and female mice were at risk for development of late-appearing tumors. In the male rats, time-adjusted analysis of the incidence of tumors was used for determining statistical significance.

In female rats, the incidence of uterine adenocarcinomas was higher in the low- and high-dose groups ($P > 0.001$) than in the pooled controls (controls 0/38, low-dose 10/33, high-dose 11/32); the incidence was also dose related ($P < 0.001$).

In male rats, no tumors occurred in the dosed groups in a significantly increased incidence.

In female mice, the incidence of all hematopoietic neoplasms (histiocytic lymphomas, lymphocytic lymphomas, or lymphocytic leukemias), taken together, was higher in the low-dose group ($P = 0.038$) and in the high-dose group ($P = 0.002$) than in the pooled controls (controls 1/45, low-dose 5/31, high-dose 9/34); the incidence was also dose related ($P = 0.002$). In addition, the inci-

dence of these tumors in the high-dose group was higher (P = 0.026) than that in the vehicle controls (0/15), and the incidence was dose related (P = 0.021) using the vehicle controls. In male mice, lymphocytic neoplasms occurred only in two low-dose and two high-dose animals.

It is concluded that under the conditions of this bioassay, ICRF-159 was carcinogenic for female Sprague-Dawley rats, producing uterine adenocarcinomas, and was also carcinogenic for female B6C3F$_1$ mice, producing lymphomas.

Synonyms: (±)bis-4,4'-(1-methyl-1,2-ethanediyl)-2,6-piperazinedione

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Positive

TR-79 Dulcin (CAS: 150-69-6)

Data from this study considered to be inconclusive and not reportable; no Technical Report issued.

TR-80 Bioassay of 1,4-Dioxane for Possible Carcinogenicity (CAS No. 123-91-1)

1,4-Dioxane, a dimer of ethylene oxide, is used extensively as an industrial solvent for lacquers, varnishes, paints, plastics, dyes, oils, waxes, resins, and cellulose acetate and as an inhibitor in chlorinated solvents. In biological and chemical laboratories, dioxane is employed as a solvent for tissue processing, liquid scintillation counting, and photochemical reactions.

A bioassay of 1,4-dioxane for possible carcinogenicity was conducted by administering the test chemical in the drinking water to Osborne-Mendel rats and B6C3F$_1$ mice.

Groups of 35 rats and 50 mice of each sex were administered 1,4-dioxane at concentrations of either 0.5% to 1.0% (v/v) in the drinking water. Because of variations in the intake of water, the doses of test chemical received by the high-dose groups were not precisely twice those received by the low-dose groups; in the male mice, the high dose was only slightly greater than the low dose. The rats were dosed for 110 weeks and the mice for 90 weeks. Matched controls consisted of 35 untreated rats and 50 untreated mice of each sex. All surviving rats were killed at 110-117 weeks and all surviving mice at 90-93-weeks.

The mean body weights of the rats and mice were not consistently affected by the administration of dioxane. Survival rates of the dosed groups of rats and female mice were lower than those of corresponding control groups, but sufficient numbers of animals were at risk for development of late-appearing tumors.

In rats, the incidence of squamous-cell carcinomas of the nasal turbinates was statistically significant in tests for dose-related trend in females (P = 0.008) and for direct comparison of high-dose with matched-control males (P < 0.001) and direct comparison of dosed with control females (P ≥ 0.003) (males: controls 0/33, low-dose 12/33, high-dose 16/34; females: controls 0/34, low-dose 10/35, high-dose 8/35). In the females, but not in the males, the incidence of hepatocellular adenomas was significant (P ≥ 0.001) in tests for dose-related trend and for direct comparison of both low- and high-dose groups with controls (controls 0/31, low-dose 10/33, high-dose 11/32).

In both male and female mice, the incidence of hepatocellular carcinomas was statistically significant (P ≥ 0.001), both in tests for dose-related trend and direct comparison of both dosed groups with controls (males: controls 2/49, low-dose 18/50, high-dose 24/47; females: controls 0/50, low-dose 12/48, high-dose 29/37). The incidences remained significant when hepatocellular adenomas were combined with hepatocellular carcinomas.

It is concluded that under the conditions of this bioassay, 1,4-dioxane induced hepatocellular adenomas in female Osborne-Mendel rats. 1,4-Dioxane was carcinogenic in both sexes of rats, producing squamous-cell carcinomas of the nasal turbinates, and in both sexes of B6C3F$_1$ mice, producing hepatocellular carcinomas.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-81 Bioassay of Trimethylphosphate for Possible Carcinogenicity (CAS No. 512-56-1)

Trimethylphosphate is an alkylating agent which has been used as a gasoline additive, a methylating agent, an intermediate for the production of polymethyl phosphates, and a flame retardant in polymers.

A bioassay of trimethylphosphate for possible carcinogenicity was conducted by administering the compound by gavage to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered trimethylphosphate in distilled water three times per week at one of two doses, either 50 or 100 mg/kg body weight for the rats and either 250 or 500 mg/kg body weight for the mice. Vehicle controls consisted of groups of 20 rats and 20 mice of each sex. The rats were dosed for 104 weeks and the mice for 103 weeks. All surviving rats were killed at week 105 and all surviving mice at week 103.

Mean body weights of dosed male and female rats and female mice were slightly lower than those of the corresponding vehicle controls throughout the study; mean body weights of the male mice were comparable to those of the vehicle controls. Survival rates of both rats and

mice were high, and adequate numbers of animals were at risk for the development of late-appearing tumors.

In male rats, the incidence of fibromas of the subcutaneous tissue was higher (P = 0.036) in the high-dose group than in the vehicle controls (control 0/20, low-dose 2/50, high-dose 9/49), and there was a dose-related trend (P = 0.006) in the incidences of these fibromas. In the female rats, no tumors occurred in the dosed groups at significantly increased incidences, compared with corresponding controls.

In the male mice, no tumors occurred in the dosed groups at significantly increased incidences, compared with controls. In the female mice, the incidence of adenocarcinomas of the endometrium was higher (P = 0.004) in the high-dose group than in the vehicle controls (controls 0/16, low-dose 7/40, high-dose 13/37), and there was a significant dose-related trend (P = 0.003) in the incidences of these adenocarcinomas.

It is concluded that under conditions of this bioassay, trimethylphosphate was carcinogenic in female $B6C3F_1$ mice, inducing adenocarcinomas of the uterus/endometrium. Trimethylphosphate was associated with the induction of benign fibromas of the subcutaneous tissue in male Fischer 344 rats. No evidence of carcinogenicity of the compound was obtained in female rats or in male mice.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Positive

TR-82 Bioassay of N-Phenyl-p-phenylenediamine for Possible Carcinogenicity (CAS No. 101-54-2)

N-phenyl-p-phenylenediamine is an industrial intermediate that is used in the production of several different chemical products. It is an intermediate for photographic chemicals, pharmaceuticals, microbicides, and other organics; it is used in the manufacture of dyes and dye reagents; and it reacts with ketones to form derivatives of p-phenylenediamine which are used as antiozonants in rubber.

A bioassay of N-phenyl-p-phenylenediamine for possible carcinogenicity was conducted by administering the test chemical in the diet to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered N-phenyl-p-phenylenediamine at one of two doses, either 600 or 1,200 ppm, for 78 weeks and were then observed for 26 additional weeks. Matched controls consisted of groups of 20 untreated rats of each sex. All surviving rats were killed at 104 weeks.

Groups of 50 mice of each sex were initially administered N-phenyl-p-phenylenediamine at one of the following doses, either 2,500 or 5,000 ppm for the males and either 5,000 or 10,000 ppm for the females, for 31 weeks. Because of toxicity of the chemical, the doses were lowered at that time and terminated at 48 weeks. The animals were then observed for 43 additional weeks. Time-weighted average doses during the period of administration were 2,057 or 4,114 ppm for the males and 3,672 or 8,170 for the females. Matched controls consisted of groups of 20 untreated mice of each sex. All surviving mice were killed at 91 weeks.

Mean body weights of the dosed rats were only slightly lower than those of the matched controls during the bioassay. Mean body weights of the dosed mice were appreciably lower than those of the matched controls, and mortality was high in the dosed groups prior to the reduction of the doses, particularly in the females. Sufficient numbers of rats and mice of each sex were at risk for the development of late-appearing tumors; however, the shortened period used for administering N-phenyl-p-phenylenediamine to the mice may not have been adequate for determining the carcinogenic potential of the test chemical in this species.

In the male and female rats, the incidences of neoplasms in the groups receiving the test chemical were not significantly different from those in the corresponding control groups.

In the male mice, the incidence of combined hepatocellular adenomas and carcinomas was significantly higher (P = 0.022) in the low-dose group than in the controls, but there was no significant dose-related trend (controls 2/20, low-dose 18/49, high-dose 10/50). Furthermore, since at this laboratory the overall historical incidences of these combined lesions in male mice have been 53/340 (15.6%) and have been as high as 7/20 (35%), these neoplasms could not be established as being compound related. Unusually extensive hepatic inflammation occurred in large numbers of the dosed males (controls 0/20, low-dose 23/49, high-dose 24/50) and in lesser numbers of the dosed females (controls 1/20, low-dose 8/49, high-dose 2/48).

It is concluded that under the conditions of this bioassay, N-phenyl-p-phenylenediamine was not carcinogenic for Fischer 344 rats or for $B6C3F_1$ mice.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-83 Bioassay of Daminozide for Possible Carcinogenicity (CAS No. 1596-84-5)

A bioassay of daminozide, a plant growth regulator, for possible carcinogenicity was conducted by administering the test chemical in the diet to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered daminozide at one of two doses, either 5,000 or 10,000 ppm, for 104 weeks, then observed for an additional week. Matched controls consisted of 20 untreated males and 20 untreated females of each species. All surviving rats and mice were killed at 105 weeks.

Mean body weights of the high-dose female mice were appreciably lower than those of the corresponding controls, while mean body weights of all other dosed groups of rats and mice were essentially unaffected. No other clinical signs related to administration of daminozide were observed. Sufficient numbers of animals in all groups of rats and mice were at risk for development of late-appearing tumors.

In the male rats, no tumors occurred at incidences that were significantly higher in dosed groups than in controls, except for interstitial-cell tumors of the testis (controls 13/20, low-dose 49/50, high-dose 47/50). These tumors occurred, however, at a high spontaneous rate (182/220) in the historical-control male rats; thus, the association of the interstitial-cell tumors with administration of the chemical is doubtful.

In the female rats, adenocarcinomas of the endometrium and leiomyosarcomas of the uterus occurred only in the dosed groups (adenocarcinomas: controls 0/19, low-dose 5/50, high-dose 3/50; leiomyosarcomas: controls 0/19, low-dose 1/50, high-dose 3/50). The incidences in the dosed groups were too low to be statistically significant; however, the low incidence of these tumors in historical-control female rats (2/220 adenocarcinoma and 0/220 leiomyosarcoma) indicate that the occurrence of these tumors in the dosed female rats was associated with the administration of daminozide.

In the male mice, there was a dose-related trend ($P = 0.008$) in the incidence of hepatocellular carcinomas; also, the incidence in the high-dose group was significant ($P = 0.020$) compared with that in the controls (controls 0/14, low-dose 7/50, high-dose 13/46). The incidence of these tumors in the historical-control male mice was, however, 21/216; thus, the association of the hepatocellular carcinomas with administration of daminozide is not clear. In female mice, only three such tumors occurred.

It is concluded that under the conditions of this bioassay, daminozide was not carcinogenic in the male Fischer 344 rats or in the female $B6C3F_1$ mice. In male $B6C3F_1$ mice, the induction of hepatocellular carcinomas may have been associated with the administration of the test chemical. Daminozide was carcinogenic in female Fischer 344 rats, inducing adenocarcinomas of the endometrium of the uterus and leiomyosarcomas of the uterus.

Synonym: succinic acid mono-2,2-dimethylhydrazide

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Equivocal
Female Mice:	Negative

TR-84 Bioassay of 2,4-Diaminoanisole Sulfate for Possible Carcinogenicity (CAS No. 615-05-4)*

2,4-Diaminoanisole sulfate, a component of many hair dyes, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer observed among workers in the dye manufacturing industry.

A bioassay of technical-grade 2,4-diaminoanisole sulfate for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. 2,4-Diaminoanisole sulfate was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The time-weighted average dietary concentrations used in the chronic bioassay were 0.12 percent for the low dose rats and 0.5 percent for the high dose rats. The dietary concentrations used for low and high dose mice were 0.12 and 0.24 percent, respectively. After a 78-week period of chemical administration, observation of the rats continued for an additional 29 weeks and observation of the mice continued for an additional 19 weeks. For each species, 49 or 50 animals of each sex were placed on test as controls.

In both species, adequate numbers of animals in all groups survived long enough to be at risk from late-developing tumors.

In high dose male and female rats groups, the proportion of animals having one or more of the following malignant follicular-cell thyroid tumors: papillary adenocarcinomas, follicular-cell carcinomas, papillary cystadenocarcinomas, or adenocarcinomas NOS, was significantly greater than the proportion in corresponding control groups. For high dose male rats the proportion of animals having either a C-cell adenoma or a C-cell carcinoma was also significantly increased.

The incidence of malignant tumors (squamous-cell carcinomas, basal-cell carcinomas, or sebaceous adenocarcinomas) of the skin and its associated glands were significantly increased among high dose rats of both sexes.

Among high dose female mice, the combined incidence of thyroid follicular-cell adenomas and carcinomas was significantly increased. Among high dose male mice, the incidence of thyroid follicular-cell adenomas was significantly increased, but no follicular-cell carcinomas were observed.

Under the conditions of this bioassay, technical-grade 2,4-diaminoanisole sulfate was carcinogenic to both sexes of both species. In Fischer 344 rats dietary administration of the chemical induced increased incidences of malignant tumors of the skin and its associated glands and malignant thyroid tumors in each sex. In $B6C3F_1$ mice, dietary administration of 2,4-diaminoanisole sulfate induced thyroid tumors in each sex.

Synonyms: 4-methoxy-1,3-benzenediamine sulfate; 4-methoxy-m-phenylenediamine sulfate; 4-MMPD; 2,4-diamino-1-methoxybenzene sulfate; 2,4-DAA sulfate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

*The technical report states that 2,4-Diaminoanisole Sulfate (CAS No. 39156-41-7) was the actual chemical tested rather than 2,4-Diaminoanisole in its pure form; therefore, the CAS number for 2,4-Diaminoanisole Sulfate is used to track this study in the NTP CHEMTRACK database.

TR-85 Bioassay of 4-Chloro-m-phenylenediamine for Possible Carcinogenicity (CAS No. 5131-60-2)

4-Chloro-m-phenylenediamine, an intermediate in the preparation of dyes, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer reported among workers in the dye manufacturing industry.

A bioassay of 4-chloro-m-phenylenediamine for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. 4-Chloro-m-phenylenediamine was administered in the feed, at either of two concentrations, to groups of 49 or 50 male and 50 female animals of each species. The dietary concentrations of 4-chloro-m-phenylediamine used in the chronic bioassay for low and high dose rats were 0.2 and 0.4 percent, respectively. The time-weighted average dietary concentrations used for low and high dose mice were 0.7 and 1.4 percent, respectively. After a 78-week period of compound administration, observation of the rats continued for an additional 17 weeks. For each species, 50 animals of each sex were placed on test as untreated controls.

In both species, adequate numbers of animals in all groups survived long enough to be at risk from late-developing tumors.

Among male rats, an increased incidence of adrenal pheochromocytomas was statistically associated with dosage of 4-chloro-m-phenylenediamine. The incidence of these tumors was significantly higher in the high dose group than in the control group.

Among female mice, there was a significant increased incidence of hepatocellular carcinomas in the low dose group and a significantly combined incidence of hepatocellular carcinomas and hepatocellular adenomas in both low and high dose groups as compared to controls.

No other neoplasms in either species were considered to be related to compound administration.

Under the conditions of this bioassay, dietary administration of 4-chloro-m-phenylenediamine was carcinogenic to the experimental animals, causing an increased incidence of hepatocellular tumors in female $B6C3F_1$ mice and an increased incidence of adrenal pheochromocytomas in male Fischer 344 rats.

Synonyms: 4-chloro-1,3-benzenediamine; 4-chlorophene-1,3-diamine; 4-chloro-1,3-phenylenediamine; C.I. 76027

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Positive

TR-86 Bioassay of 1,2-Dibromoethane for Possible Carcinogenicity (CAS No. 106-93-4)

1,2-Dibromoethane, a volatile saturated brominated hydrocarbon, is used principally as a lead scavenger in tetra-alkyl lead gasoline and antiknock preparations but also as a soil and grain fumigant, a chemical intermediate, and a solvent.

A bioassay for possible carcinogenicity of technical-grade 1,2-dibromoethane was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. 1,2-Dibromoethane in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The time-weighted average high and low doses of 1,2-dibromoethane used in the chronic bioassay were, respectively, 41 and 38 mg/kg/day for male rats, 39 and 37 mg/kg/day for female rats and 107 and 62 mg/kg/day for mice of both sexes. For each species 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil with the same frequency that dosed animals were gavaged with 1,2-dibromoethane mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

There was a positive association between increased dosage and accelerated mortality in rats and mice of both sexes. All surviving dosed male rats were sacrificed in week 49 and all surviving dosed female rats were sacrificed after 61 weeks of compound administration. All male mice and high dose female mice died or were sacrificed by week 78, while the low dose mice were observed for an additional 37 weeks after a 53-week period of chemical administration.

In rats squamous-cell carcinomas of the forestomach were observed in 45/50, 33/50, 40/50 and 29/50 of the low dose males, high dose males, low dose females and high dose females, respectively, while none were observed in controls. Each of these incidences was statistically significant. These lesions were seen as early as week 12 in rats and week 24 in mice; they invaded locally and eventually metastasized. Increased incidences of hepatocellular carcinomas were observed in dosed rats, but the incidence of this neoplasm was significant only in females. Increased incidences of hemangiosarcomas were observed in each dosed rat group, but was statistically significant only in males, where they appeared as early as week 26.

Early development of squamous-cell carcinomas which invaded and metastasized was also observed among mice. Squamous-cell carcinomas were found in 45/50, 29/49, 46/49 and 28/50 of the low dose males, high dose males, low dose females, and high dose females, respectively, but none were found in controls. Each of these incidences was statistically significant. Incidences of alveolar/bronchiolar adenomas were significant for male and female dosed mice.

Under the conditions of this bioassay, 1,2-dibromoethane was carcinogenic to Osborne-Mendel rats and $B6C3F_1$ mice. The compound induced squamous-cell carcinomas of the forestomach in rats of both sexes, hepatocellular carcinomas in female rats, and hemangiosarcomas in male rats. In mice of both sexes the compound induced squamous-cell carcinomas of the forestomach and alveolar/bronchiolar adenomas.

Synonyms: DBE; sym-dibromoethane; ethylene dibromide; EDB; glycol dibromide

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

Note: 1,2-dibromoethane was subsequently studied by inhalation in F344 rats and $B6C3F_1$ mice (See TR-210, reported 1982).

TR-87 1,3-Dichloro-5,5-Dimethylhydantoin (Ethylene Dibromide) (CAS: 118-52-5)

Data from this study considered to be inconclusive and not reportable; no Technical Report issued.

TR-88 Bioassay of 1H-Benzotriazole for Possible Carcinogenicity (CAS No. 95-14-7)

1H-Benzotriazole is an anticorrosive chemical used primarily on copper, but also on iron, steel, cadmium, chromium, zinc, and silver-nickel alloys.

A bioassay of 1H-benzotriazole for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered 1H-benzotriazole at one of two time-weighted average doses, either 6,700 or 12,100 ppm, for 78 weeks. Except for five control and five high-dose rats of each sex, which were killed at week 78, all animals surviving at that time were observed for 26-27 additional weeks. Controls consisted of groups of 50 untreated rats of each sex and were observed for 105-106 weeks. All rats surviving to weeks 104-106 were then killed.

Groups of 50 mice of each sex were administered 1H-benzotriazole at one of two time-weighted average doses, either 11,700 or 23,500 ppm, for 104 weeks, then observed for 2 additional weeks. Controls consisted of groups of 50 untreated mice of each sex and were observed for 109 weeks. All mice surviving to weeks 106-109 were then killed.

Mean body weights of the dosed male and female rats and mice were lower than those of the corresponding controls throughout most of the bioassay. Survival of animals in dosed and control groups of both rats and mice was at least 60%, and sufficient numbers of animals were at risk for development of late-appearing tumors.

In male rats, neoplastic nodules of the liver occurred at a statistically significant incidence ($P = 0.024$) in the high-dose group when compared with the control group (controls 0/48, low-dose 0/46, high-dose 5/45 [11%]). The incidence of this tumor in control Fischer 344 rats used in similar bioassays of other test chemicals at the same laboratory has varied from 0 to 11%, with 2/13 historical-control groups having incidences of 10-11%. Since the incidence in the high-dose groups is no higher than has been observed in some control groups, these tumors cannot be clearly associated with administration of the test chemical.

Brain tumors occurred in three dosed male rats, in one dosed female rat, and in none of the controls. The occurrence of this rare tumor in dosed animals of each sex is suggestive of, but not considered as sufficient evidence of, carcinogenicity.

In female rats, the incidence of endometrial stromal polyps in the low-dose group was significantly higher ($P = 0.010$) than that in the corresponding controls (controls 2/48, low-dose 10/45, high-dose 8/50). However, the incidence in the high-dose group was not significant, and when the incidences of endometrial stomal polyps and endometrial stromal sarcomas were combined, they were not significant in either the low- or high-dose groups. Thus, these tumors cannot be associated with administration of the chemical.

In male mice, no tumors occurred in dosed groups at incidences that were significantly higher than those in controls.

In female mice, alveolar/bronchiolar carcinomas occurred at a statistically significant incidence ($P = 0.001$) only in the low-dose groups when compared with the control group (controls 0/49, low-dose 9/49 [18%], high-dose 3/59 [6%]). The incidence in the high-dose group was not significant, and the data did not show a dose-related trend. It should be noted that the incidence of these tumors in control $B6C3F_1$ female mice from other bioassays at this laboratory has varied from 0 to 7%, with a mean of 4%. Therefore, the occurrence of this tumor in female mice cannot be clearly related to the administration of the test chemical.

In female $B6C3F_1$ mice there was an increased incidence of alveolar/bronchiolar carcinomas, suggesting a possible carcinogenic effect of 1H-benzotriazole. In Fischer 344 rats there was an increased incidence of brain tumors, suggesting a possible carcinogenic effect.

However, there was no convincing evidence that under the conditions of this bioassay 1H-benzotriazole was carcinogenic in B6C3F$_1$ mice or Fischer 344 rats of either sex.

Synonym: 1,2,3-benzotriazole

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Equivocal
Male Mice:	Negative
Female Mice:	Equivocal

TR-89 Bioassay of *o*-Anisidine Hydrochloride for Possible Carcinogenicity (CAS No. 134-29-0)

o-Anisidine is used chiefly in the manufacture of dyes, one method being the diazotization of *o*-anisidine and coupling with other aromatic amines or phenols to yield a large number of the azo dyes.

A bioassay of *o*-anisidine hydrochloride for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 55 rats of each sex and 55 mice of each sex were administered *o*-anisidine hydrochloride at one of the following doses, either 5,000 or 10,000 ppm for rats and either 2,500 or 5,000 ppm for mice, for 103 weeks, then observed for 1 or 2 additional weeks. Controls consisted of groups of 55 untreated rats of each sex and 55 untreated mice of each sex. All surviving rats were killed at 103-107 weeks, and all surviving mice at 104 or 105 weeks.

Mean body weights of the dosed male and female rats and mice were lower than those of the corresponding controls throughout the bioassay. Bloody exudates and stained fur in the urogenital area were noted in many dosed animals. Sufficient numbers of animals were at risk in the mice, but not in the rats, for development of late-appearing tumors; however, survival in the rats was 80% or more at week 52.

Transitional-cell carcinomas or papillomas of the urinary bladder occurred at statistically significant incidences ($P<0.001$) in the low- and high-dose groups of rats (males: controls 0/51, low-dose 52/54, high-dose 52/52; females: controls 0/49, low-dose 1/51, high-dose 22/50); the incidences also had significant dose-related trends ($P<0.001$) in both species. These lesions were observed as early as week 36 in female rats, week 40 in male rats, and week 45 in male mice. Transitional-cell carcinomas of the pelvis of the kidney occurred with a significant dose-related trend ($P=0.005$) in the male rats, and the incidence in the high-dose group was significantly higher ($P=0.006$) than that in the control group (controls 0/53, low-dose 3/55, high-dose 7/53); all rats having this tumor also has a transitional-cell carcinoma of the urinary bladder. Only one animal in the control groups of rats or mice had any tumor of the urinary system (a transitional-cell papilloma of the pelvis of the kidney in a male mouse).

Follicular-cell tumors of the thyroid (carcinomas, cystadenocarcinomas, adenomas, cystadenomas, and papillary cystadenomas) occurred at statistically significant incidences ($P\leq 0.005$) in low- and high-dose groups of male rats (controls 0/53, low-dose 7/40, high-dose 6/40); the incidences also had a dose-related trend ($P=0.009$). These tumors did not occur at significant incidences in dosed groups of female rats.

It is concluded that under the conditions of this bioassay, *o*-anisidine hydrochloride was carcinogenic for Fischer 344 rats and B6C3F$_1$ mice, inducing transitional-cell carcinomas or papillomas of the bladder in both rats and mice and in both sexes of each species, transitional-cell carcinomas of the pelvis of the kidney in male rats, and follicular-cell tumors of the thyroid in male rats.

Synonyms: 2-methoxyaniline; Fast Red BB Base

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-90 Bioassay of Dicofol for Possible Carcinogenicity (CAS No. 115-32-2)

Dicofol, a synthetic organochlorine acaricide, was selected for bioassay by the National Cancer Institute because it is an alcohol analog of the known tumorigen DDT. Its widespread use on edible crops was also an important factor in its selection for testing.

A bioassay of technical-grade dicofol for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. Dicofol was administered in the feed, at either of two concentrations, to groups of 50 males and 50 females of each species. The high and low time-weighted average concentrations of dicofol were, respectively, 942 and 471 ppm for male rats, 760 and 380 ppm for female rats, 528 and 264 ppm for male mice, and 243 and 122 ppm for female mice. For each species, 20 animals of each sex were placed on test as controls. The period of compound administration was 78 weeks, followed by 34 weeks of observation in rats and 14 or 15 weeks in mice.

There was no statistically significant positive association between dietary concentration and mortality in either sex or species.

Hepatocellular carcinomas in dosed male mice were the only neoplasms that occurred in any dosed group of either species in statistically significant increased incidences when compared to controls. The Cochran-Armitage test as well as the Fischer exact test for both the high and low dose groups supported the association

between compound administration and increased incidences of this tumor in the male mice. No increase in hepatocellular carcinomas was observed in dosed female mice.

Under the conditions of this bioassay, technical-grade dicofol was carcinogenic in male $B6C3F_1$ mice, causing hepatocellular carcinomas. No evidence for carcinogenicity was obtained for this compound in Osborne-Mendel rats of either sex or in female $B6C3F_1$ mice.

Synonyms: 4-chloro-α-(4-chlorophenyl)-α-(trichloromethyl)benzenemethanol; 1,1-bis(p-chlorophenyl)- 2,2,2-trichloroethanol; 4,4'-dichloro-α-trichloromethyl) benzhydrol; 2,2,2-trichloro-1,1-di-(4-chlorophenyl) ethanol

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Negative

TR-91 Bioassay of Clonitralid for Possible Carcinogenicity (CAS No. 1420-04-8)

Clonitralid, a powerful molluscicide and lamprey killer, was selected for bioassay by the National Cancer Institute because of the large potential for human exposure resulting from the direct application of the compound for control of sea lamprey larvae in tributaries to the Great Lakes and the widespread application of clonitralid for the control of water snails.

A bioassay for possible carcinogenicity of clonitralid was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Clonitralid was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The time-weighted average high and low dietary concentrations of clonitralid were, respectively, 28,433 and 14,216 ppm for rats and 549 and 274 ppm for mice. After a 78-week period of compound administration, there was an additional observation period of 32 to 33 weeks for rats and 13 to 14 weeks for mice.

Adequate numbers of male rats, female rats, and female mice survived long enough to be at risk from late-developing tumors. Because of inadequate survival among male mice, however, results obtained from observation of the male mouse groups cannot be considered conclusive.

The incidences of mammary adenocarcinomas in treated female rats were not significantly higher than the incidences observed in control female rats. However, the incidences of this lesion in dosed female rats were greater than or equal to 22 percent, while the highest incidence observed in 15 control groups at this laboratory was only 10 percent with a mean incidence of 2.6 percent. The occurrence in high dose female rats (2/45) of carcinomas in the glandular portion of the stomach with metastases to other sites was not statistically significant. This incidence, however, is much greater than the historical control incidence and suggests an association between administration of clonitralid and the development of these tumors.

No statistically significant increased tumor incidences were observed among male rats or mice of either sex dosed with clonitralid.

Under the conditions of this bioassay, there was no convincing evidence that clonitralid was carcinogenic to Osborne-Mendel rats or to female $B6C3F_1$ mice. Poor survival of male mice did not permit an evaluation of carcinogenicity in these animals.

Synonyms: 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide compound with 2-aminoethanol (1:1); Clonitralide; Bayer 25648; Bayer 73; SR 73

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Equivocal
Male Mice:	Inadequate Study
Female Mice:	Negative

TR-92 Bioassay of Hydrazobenzene for Possible Carcinogenicity (CAS No. 122-66-7)

Hydrazobenzene is a hydrazine derivative selected for bioassay by the National Cancer Institute because of the documented carcinogenicity of the parent compound hydrazine and of certain substituted hydrazines. Treatment of hydrazobenzene with hot mineral acid results in the production of benzidine (the so-called "benzidine rearrangement") and hydrazobenzene finds application in the dye manufacturing industry as a precursor of this important dye intermediate and potent carcinogen.

A bioassay of technical-grade hydrazobenzene for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. Hydrazobenzene was administered in the feed, at either of two concentrations, to groups of 50 male and 47 to 50 females animals of each species. The time-weighted average dietary concentrations used in the rat bioassay were 0.008, 0.03, 0.004, and 0.01 percent for low dose males, high dose males, low dose females, and high dose females, respectively. The time-weighted average dietary concentrations used in the mouse bioassay were 0.008, 0.04, 0.004, and 0.04 percent for low dose males, high dose males, low dose females, and high dose females, respectively. After a 78-week period of compound administration, observation of the rats continued for an additional 28 to 30 weeks and observation of the mice continued for an additional 17 or 18 weeks. For each species, 47 to 50 animals of each sex were placed on test as controls.

In both, species, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-appearing tumors.

The incidence of hepatocellular carcinomas was significantly increased in dosed male rats and the incidence of neoplastic nodules of the liver was significantly increased in dosed female rats. A significant increase in the combined incidence of squamous-cell carcinomas or squamous-cell papillomas of the Zymbal's gland, the ear canal, or the skin of the ear was observed among high dose male rats. A significant increase in mammary adenocarcinomas was observed among dosed female rats.

The incidence of hepatocellular carcinomas was significantly increased among female mice, but no significant increase in liver tumors was observed among male mice.

Under the conditions of this bioassay, hydrazobenzene was carcinogenic to Fischer 344 rats of both sexes, causing increased incidences of hepatocellular carcinoma and Zymbal's gland squamous-cell neoplasms in male rats, neoplastic nodules of the liver in female rats, and mammary adenocarcinomas in female rats. Hydrazobenzene was also carcinogenic to female B6C3F$_1$ mice, causing an increased incidence of hepatocellular carcinomas. The compound was not carcinogenic to male B6C3F$_1$ mice.

Synonym: 1,2-diphenylhydrazine

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Positive

TR-93 Bioassay of 3-Amino-9-ethylcarbazole Hydrochloride for Possible Carcinogenicity (CAS No. 132-32-1)*

3-Amino-9-ethylcarbazole, is an aromatic amine dye intermediate that has been used industrially in the manufacture of C.I. Pigment Violet 23 and C.I. Direct Blue 108.

A bioassay of 3-amino-9-ethylcarbazole (hydrochloride) for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice. Both the free amine and the hydrochloride salt were used.

Groups of 50 rats of each sex and 50 mice of each sex were administered the test chemical at one of two doses, either 800 or 2,000 ppm for rats and either 800 or 1,200 ppm for mice, for 78 weeks. The rats were then observed for an additional 26-29 weeks, and the mice for an additional 16-17 weeks. Controls consisted of groups of 50 untreated rats of each sex and 50 untreated mice of each sex; separate controls were used for the groups of animals administered the different doses. All surviving rats were killed at 104-110 weeks; all surviving mice were killed at 94-97 weeks.

Since the suppliers of the low-dose rats and mice differed from those of the corresponding low-dose controls, while the suppliers for the high-dose rats and mice were the same as those of the corresponding high-dose controls, comparions of the high-dose groups with their corresponding controls were the most appropiate. Furthermore, since the low-dose animals did not receive the same regimen of administration of the test compound as that received by the high-dose animals, and since tests using the low-dose groups were not performed concurrently with those using the high-dose groups, analyses of the dose-related trends were not possible. Although the interpretation of results of the study was based primarily on comparisons of high-dose groups with their respective controls, the results obtained with the low-dose groups, regardless of the indicated complicating factors, supported the interpretation.

Neoplasms of the liver were observed in significant incidences in rats and mice of both sexes. In male rats, hepatocellular carcinomas alone were significantly higher($P \leq 0.020$) in both the low- and high-dose groups. When neoplastic nodules of the liver were combined with hepatocellular carcinomas, the combination occurred at significant incidences ($P \leq 0.012$) in the low- and high-dose male rats and in the high-dose female rats (males: low-dose controls 0/36, low-dose 12/42; high-dose controls 1/48, high-dose 22/48; females: high-dose controls 0/50, high-dose 6/48). Hepatocellular carcinomas alone similarly occurred at significant incidences ($P<0.001$) in the low- and high-dose male and female mice (males: low-dose controls 7/48, low-dose 32/44; high-dose controls 6/44, high-dose 41/49; females: low-dose controls 1/47, low-dose 36/43; high-dose controls 1/45; high-dose 43/49).

Papillomas or carcinomas of the integumentary system occurred at significant incidences ($P \leq 0.013$) in both the low- and high-dose male rats (low-dose controls 0/36, low-dose 8/44; high-dose controls 0/48, high-dose 6/48); these tumors also occurred in low- and high-dose female rats, but not at incidences high enough to be statistically significant (low-dose control 0/39, low-dose 4/44; high-dose controls 0/50, high-dose 4/49).

Carcinomas of the Zymbal's glands of the ear occurred at significant incidences ($P \leq 0.045$) in the low- and high-dose male and female rats (males: low-dose controls 0/36, low-dose 5/44; high-dose controls 0/48, high-dose 7/48; females: low-dose controls 0/39, low-dose 10/44; high-dose controls 0/50, high-dose 12/49). These tumors were first observed as early as week 47 on study.

Adenocarcinomas of the uterus or endometrium occurred at a significant incidence ($P = 0.002$) only in the high-dose female rats (high-dose controls 1/50, high-dose 11/49); these tumors also occurred in the low-dose females, but not at an incidence high enough to be statistically significant (low-dose controls 4/38, low dose 11/43).

It is concluded that under the conditions of this bioassay, 3-amino-9-ethylcarbazole (hydrochloride) was carcinogenic for the liver, inducing hepatocellular carcinomas in Fischer 344 rats and B6C3F$_1$ mice of both sexes. Other tumors induced in the rats were carcinomas

or papillomas of the integumentary system in males, carcinomas of the Zymbal's gland of the ear in males and females, and adenocarcinomas of the uterus.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

*The technical report states that 3-Amino-9-ethylcarbazole Hydrochloride (CAS No. 6109-97-3) was the actual chemical tested rather than 3-Amino-9-ethylcarbazole in its pure form; therefore, the CAS number for 3-Amino-9-ethylcarbazole Hydrochloride is used to track this study in the NTP CHEMTRACK database.

TR-94 Bioassay of 4-Amino-2-nitrophenol for Possible Carcinogenicity (CAS No. 119-34-6)

4-Amino-2-nitrophenol is used as an industrial dye intermediate, and as a constituent of "semi-permanent" hair dyes.

A bioassay of 4-amino-2-nitrophenol for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered 4-amino-2-nitrophenol at one of two doses, either 1,250 or 2,500 ppm, for 103 weeks. Matched controls consisted of groups of 20 untreated rats and 20 untreated mice of each sex. All dosed and matched-control groups of each species and sex were killed at 105 weeks.

Mean body weights of dosed rats of each sex were not appreciably affected by administration of the 4-amino-2-nitrophenol, and mean body weights of dosed mice of each sex were only slightly lower than those of corresponding matched controls. Survival of neither rats nor mice was affected by the test chemical, and sufficient numbers of animals in dosed and control groups were at risk for the development of late-appearing tumors. Since both male and female mice receiving 4-amino-2-nitrophenol had little or no depression in mean weights and their survival was comparable to that of controls, they may have been able to tolerate a higher dose.

In rats, transitional-cell carcinomas of the urinary bladder showed a dose-related trend in the males ($P<0.001$) and occurred at a significantly higher incidence ($P=0.018$) in the high-dose males than in the matched-control males (controls 0/15, low-dose 0/46, high-dose 11/39 [28%]). Carcinomas of the bladder also occurred in one low-dose female and two high-dose females, but in none of the control females. Transitional-cell papillomas of the bladder occurred in two additional high-dose males, and transitional-cell hyperplasia of the bladder occurred in four additional high-dose males, but neither lesion occurred in control males. No tumors of the bladder were found among 220 male and 220 female historical-control rats at this laboratory.

In mice, no tumors occurred in dosed groups of males or females at incidences that were significantly higher than those in the corresponding matched-control groups.

Deposition of pigment occurred in the lamina propria of the small intestine in at least 91% of the animals in the dosed groups of rats and in at least 89% of the animals in the dosed groups of mice, but in none of the control groups of either species.

It is concluded that under the conditions of the bioassay, 4-amino-2-nitrophenol was carcinogenic for male Fischer 344 rats, inducing transitional-cell carcinomas of the urinary bladder; the transitional-cell carcinomas of the urinary bladder observed in three dosed female rats may also have been associated with administration of the 4-amino-2-nitrophenol. The test chemical was not carcinogenic for male or female B6C3F$_1$ mice at the doses tested.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Equivocal
Male Mice:	Negative
Female Mice:	Negative

TR-95 Bioassay of 3-(Chloromethyl) pyridine Hydrochloride for Possible Carcinogenicity (CAS No. 6959-48-4)

3-(Chloromethyl)pyridine hydrochloride is an intermediate that has been proposed for use in the synthesis of agricultural, pharmaceutical, and veterinary chemicals.

A bioassay of 3-(chloromethyl)pyridine hydrochloride for possible carcinogenicity was conducted by administering the test chemical by gavage to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered 3-(chloromethyl)pyridine hydrochloride in a vehicle of distilled water three times per week at one of the following doses, either 75 or 150 mg/kg body weight for the rats and either 100 or 200 mg/kg body weight for the mice. The low-dose rats were dosed for 103 weeks and the low-dose mice for 102 weeks. Because of early deaths in the high-dose animals, the high-dose rats were dosed for only 83 weeks and the high-dose mice for only 81 weeks. Controls consisted of groups of 20 rats and 20 mice of each sex which were administered the vehicle only for 104 weeks. All surviving rats and mice were killed at 104 weeks.

Mean body weights of the male and female rats were lower in the dosed groups than in the corresponding control groups, and the depressions in weight were dose related. At the termination of the administration of the test chemical to the high-dose groups of rats, the mean

body weights of these groups recovered rapidly. The mean body weights of the male mice were unaffected by the administration of the chemical; those of the females were only slightly affected. Mortality was generally higher in the dosed groups of rats and mice than in the corresponding control groups and was dose related in all tests except those using the female mice; however, sufficient numbers of animals of each species and sex were at risk for the development of late-appearing tumors.

In rats, proliferative squamous-cell lesions of the forestomach were observed in the dosed males (carcinomas: high-dose 1/50; papillomas: low-dose 1/47; high-dose 2/50; hyperplasias: low-dose 1/47, high-dose 2/50) and the dosed females (carcinomas: high-dose 1/48), but not in the male or female vehicle controls. The results of the Fischer exact test were not significant for squamous-cell papillomas or carcinomas. However, comparison of the incidence of these tumors in the high-dose males with that in 99 historical vehicle controls shows that the probability that three or more such tumors did not occur by chance, given that none have been observed in the controls in this laboratory, is $P = 0.014$.

In mice, squamous-cell papillomas or carcinomas of the forestomach occurred in the low- and high-dose groups of each sex, but not in the corresponding control groups. The incidence in the high-dose males was significantly higher ($P = 0.025$) than that in the control males (males: vehicle controls 0/19; low-dose 2/43; high dose 10/47 [21%]; females: vehicle controls 0/19, low-dose 1/45, high-dose 5/48 [10%]). Comparison of the incidences of these tumors in the high-dose males and females with those observed in the corresponding control groups of 100 historical vehicle controls of each sex shows that the probability that their occurrence was not due to chance is $P < 0.001$. Also, a life-table analysis of the incidence in males indicated a significant ($P = 0.003$) increase in tumors over the period of observation (58 weeks to 104 weeks) in relation to an increase in dose.

Although the incidence of squamous-cell papillomas and carcinomas in male and female rats was significant only in males compared with historical vehicle controls, these tumors are of the same type as those appearing at the same site in male and female mice. Because these tumors are rare and not found in controls, and because they were found in dosed animals of both species, they are considered to be related to administration of the test chemical by gavage.

It is concluded that under the conditions of this bioassay, 3-(chloromethyl)pyridine hydrochloride was carcinogenic in male Fischer 344 rats and $B6C3F_1$ mice of both sexes, producing papillomas and carcinomas at the site of topical application, the stomach.

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Equivocal
Male Mice:	Positive
Female Mice:	Positive

TR-96 Bioassay of Coumaphos for Possible Carcinogenicity (CAS No. 56-72-4)

Coumaphos is an organophosphorus pesticide that was developed in Germany by G. Schrader. Coumaphos, which has a relatively low mammalian toxicity in relation to the other organophosphates, is used principally on livestock and poultry to control ectoparasites.

A bioassay of coumaphos for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered the coumaphos in the diet at one of two doses, either 10 or 20 ppm, for 103 weeks and then observed for 0-1 additional weeks. Matched controls consisted of groups of 25 untreated animals of each species and sex. All surviving animals were killed at 103-105 weeks.

Mean body weights of the dosed female rats were lower than those of corresponding controls, while mean body weights of dosed male rats and of dosed male and female mice were essentially unaffected. No clinical signs that are typical of organophosphorus poisoning were reported in either rats or mice. Survival of the rats and mice was not affected by administration of the test chemical. The test animals may have been able to tolerate higher doses. Sufficient numbers of animals in all groups of the rats and mice were at risk for the development of late-appearing tumors.

In both rats and mice, no tumors occurred in the dosed groups of either sex at incidences that were significantly higher than those in corresponding control groups.

It is concluded that under the conditions of this bioassay, coumaphos was not carcinogenic for either F344 rats or $B6C3F_1$ mice.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-97 Bioassay of Titanium Dioxide for Possible Carcinogenicity (CAS No. 13463-67-7)

Titanium dioxide is a white pigment possessing great covering or opacifying power. It exists in three crystalline forms: anatase, brookite, and rutile, but only the anatase variety is used as a food color additive.

A bioassay of titanium dioxide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered titanium dioxide in the diet at one of two doses, either 25,000 or 50,000 ppm, for 103 weeks and then observed for 1 additional week. Matched controls

consisted of 50 untreated rats of each sex and 50 untreated mice of each sex. All surviving rats and mice were killed at 104 weeks.

Administration of the titanium dioxide had no appreciable effect on the mean body weights of rats or mice of either sex. With the exception of white feces, there was no other clinical sign that was judged to be related to the administration of titanium dioxide. Survival of the rats and the male mice at the end of the bioassay was not affected by the test chemical; mortality in female mice was dose related. Sufficient numbers of dosed and control rats and mice of each sex were at risk for development of late-appearing tumors.

In the female rats, C-cell adenomas or carcinomas of the thyroids occurred at incidences that were dose related ($P = 0.013$), but were not high enough ($P = 0.043$ for direct comparison of the high-dose group with the control group) to meet the level of $P = 0.025$ required by the Bonferroni criterion (controls 1/48, low-dose 0/47, high-dose 6/44). Thus, these tumors of the thyroid were not considered to be related to the administration of the test chemical.

In male and female mice, no tumors occurred in dosed groups at incidences that were significantly higher than those for corresponding control groups.

It is concluded that under the conditions of this bioassay, titanium dioxide was not carcinogenic by the oral route for Fischer 344 rats or $B6C3F_1$ mice.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-98 Bioassay of dl-Menthol for Possible Carcinogenicity (CAS No. 89-78-1)*

Menthol is a naturally occurring monocyclic terpene found in the oils of the mint tree *Mentha arvensis*. Menthol is well known for its cooling effects and its mint flavor and odor, which are the basis of the majority of its uses. The single largest use for menthol is probably in cigarettes. A survey of pharmaceutical products indicates that menthol is formulated in over-the-counter rubs and liniments (2-10% concentrations), antipruritic lotions, nasal sprays, expectorants, mouthwashes and sprays, cough drops, and foot powders.

A bioassay of dl-menthol for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered dl-menthol at one of the following doses, either 3,750 or 7,500 ppm for the rats and either 2,000 or 4,000 ppm for the mice, for 103 weeks, then observed for 1 or 2 additional weeks. Matched controls consisted of 50 untreated rats of each sex and 50 untreated mice of each sex. All surviving rats were killed at 105 weeks and all surviving mice at 104 weeks.

Mean body weights of dosed rats and mice were only slightly lower than those of corresponding controls. No other clinical signs related to administration of the dl-menthol were noted in the dosed groups of animals. A dose-related trend in mortality was observed only in the female mice. Survival at the end of the bioassay was at least 62% in all dosed and control groups of animals of each species, and sufficient numbers of animals were at risk for the development of late-appearing tumors.

In male rats, no tumors occurred at incidences which were considered to be related to the administration of dl-menthol.

In female rats, no tumors occurred at higher incidences in the dosed groups than in the control groups. Fibroadenomas of the mammary gland occurred at lower incidences in the low-dose (10/49) and high-dose (7/49) groups than in the control group (20/50), and alveolar/bronchiolar adenomas or carcinomas of the lung occurred only in the controls (3/50).

In mice of either sex, no tumors occurred in dosed groups at incidences that were significantly different from those for corresponding control groups.

It is concluded that under the conditions of this bioassay, dl-menthol was not carcinogenic for either Fischer 344 rats or $B6C3F_1$ mice.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

*The Chemical Abstracts Service (CAS) Registry Number used to track this bioassay in the NTP CHEMTRACK database is 15356-70-4 which is determined to best define the material used in the conduct of this bioassay.

TR-99 Bioassay of Phenazopyridine Hydrochloride for Possible Carcinogenicity (CAS No. 136-40-3)

Phenazopyridine hydrochloride is the generic name for an azo dye which has been used for 40 years as an analgesic drug to reduce pain associated with urinary tract infections. It is marketed both alone and in combination with the sulfonamide urinary tract antiseptics.

A bioassay of phenazopyridine hydrochloride for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 35 rats and 35 mice of each sex were administered phenazopyridine hydrochloride at one of

the following doses, either 3,700 or 7,500 ppm for rats and either 600 or 1,200 ppm for mice. The rats were administered the chemical for 78 weeks, then observed for 26 or 27 additional weeks; the mice were administered the chemical for 80 weeks, then observed for 25-27 additional weeks. Matched controls consisted of 15 untreated rats and 15 untreated mice of each sex. All surviving rats were killed at 104 or 105 weeks, all surviving mice at 105-107 weeks.

Mean body weights of the dosed rats and mice of each sex were consistently lower than those of corresponding control animals, and the depressions in mean body weight were dose related. Mortality in the groups of rats and mice did not, however, show dose-related trends, and sufficient numbers of animals of both dosed and control groups were at risk for the development of late-appearing tumors.

In male rats, adenomas or adenocarcinomas of the large intestine (colon or rectum) occurred at incidences having a significant dose-related trend ($P = 0.015$). The direct comparison of the incidences in each of the dosed groups with that in the control group was not significant (controls 0/14, low-dose 4/34, high-dose 8/35). In the females, 3/33 low-dose and 5/32 high-dose animals, but no control animals, had this tumor. In addition, sarcomas were observed in the colon of one low-dose male and one high-dose female. The laboratory historical records showed no incidence of adenomas or adenocarcinomas of the large intestine in 260 females and only one adenomatous polyp in 260 males. Assuming a spontaneous incidence of 1/261 (0.038%) and a binomial distribution of such tumors, the occurrence seen in the male and female high-dose groups are both significantly ($P < 0.001$) different from the expected value. Thus, these tumors are considered to be related to administration of the test chemical.

In female mice, the combined incidence of hepatocellular adenomas and carcinomas showed a significant dose-related trend ($P = 0.002$), and the incidence in the high-dose group was significant ($P = 0.003$) when compared with that in the control group (controls 2/15, low-dose 11/34, high-dose 19/32). The incidence of hepatocellular carcinomas, considered alone, also was significant in female mice, showing a dose-related trend ($P = 0.010$) and a P value of 0.039 for the comparison of the high-dose group with the control group. In the males, the combined incidence of hepatocellular adenomas and carcinomas was not significant.

It is concluded that under the conditions of this bioassay, phenazopyridine hydrochloride was carcinogenic in Fischer 344 rats, inducing adenocarcinomas of the colon in both males and females. Although phenazopyridine hydrochloride was not carcinogenic in male $B6C3F_1$ mice, the chemical was carcinogenic in females, inducing hepatocellular adenomas and carcinomas.

Synonym: 2,6-diamino-3-(phenylazo)-pyridine monohydrochloride

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Positive

TR-100 Bioassay of Cupferron for Possible Carcinogenicity (CAS No. 135-20-6)

Cupferron, an N-nitroso hydroxylamine derivative used primarily as a reagent in analytical chemistry, was selected for bioassay by the National Cancer Institute because of the suspected carcinogenicity of nitrosamines.

A bioassay of cupferron for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. Cupferron was administered in the feed, at either of two concentrations, to groups of 49 or 50 male and 50 female animals of each species. The time-weighted average high and low dietary concentrations of cupferron were, respectively, 0.30 and 0.15 percent for male and female rats, and 0.4 and 0.2 percent for male and female mice. After a 78-week period of compound administration, observation of the rats continued for an additional period of up to 28 weeks and observation of the mice continued for an additional period of up to 18 weeks.

For each species, 50 animals of each sex were placed on test as controls and fed only the basal diet.

Among both sexes of rats and mice there was a significant positive association between the dose of cupferron administered and mortality; however, in all groups of animals sufficient numbers survived long enough to establish the carcinogenicity of this compound.

There were significant positive associations between the concentrations of cupferron administered to male and female rats and the incidences of: squamous-cell carcinomas of the forestomach, hepatocellular carcinomas and neoplastic nodules, and hemangiosarcomas. When a binomial distribution and a spontaneous incidence rate corresponding to the appropriate historical control incidence were assumed, the incidences of auditory sebaceous gland neoplasms in female rats and female mice were significant. There were significant positive associations between the concentrations administered and the incidences of hepatocellular carcinomas in female mice, the incidences of hemangiosarcomas in both sexes of mice, and the incidence of Harderian gland adenomas in both sexes of mice.

Under the conditions of this bioassay cupferron was carcinogenic in Fischer 344 rats, causing hemangiosarcomas, hepatocellular carcinomas, and squamous-cell carcinomas of the forestomach in males and females as well as carcinomas of the auditory sebaceous gland in females. The chemical was also carcinogenic in $B6C3F_1$ mice, causing hemangiosarcomas in males and hepatocellular carcinomas, carcinomas of the auditory sebaceous gland, a combination of hemangiosarcomas

and hemangiomas, and adenomas of the Harderian gland in females.

Synonyms: N-hydroxy-N-nitroso-benzeneamine, ammonium salt; N-nitroso-N-phenyl-hydroxylamine, ammonium salt; ammonium nitrosophenylhydroxylamine

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-101 Bioassay of Formulated Fenaminosulf for Possible Carcinogenicity (CAS No. 140-56-7)

Fenaminosulf, an aromatic diazo compound used exclusively as a fungicide, was selected for bioassay by the National Cancer Institute because of conflicting reports concerning its ability to induce hepatomas in rats.

A bioassay of formulated fenaminosulf for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F$_1$ mice. Fenaminosulf was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The time-weighted average high and low dietary concentrations of fenaminosulf were, respectively, 0.10 and 0.05 percent for rats, 0.19 and 0.10 percent for male mice, and 0.10 and 0.05 percent for female mice. After a 78-week period of compound administration, observation of the rats continued for up to an additional 31 weeks and observation of the mice continued for up to an additional 19 weeks.

Fifty male mice and 50 rats of each sex were placed on test as controls and fed only the basal diet. For female mice, 50 animals served as controls for the high dose group and 50 as controls for the low dose group.

For female rats there was no significant association between fenaminosulf dosage and mortality and, if the 21 male rats that died in the first two weeks of the bioassay were excluded from consideration, the same was true for male rats. For both male and female mice there was a significant positive association between dosage and mortality. In all groups of both species, however, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors.

No convincing, statistically significant positive associations were demonstrated between administration and the incidence of neoplasms in either sex of either species. An increased incidence of necrosis and mineralization of the tubular cells of the renal papilla occurred in treated rats and mice. These nonneoplastic lesions were not present in control animals of either species.

Under the conditions of this bioassay, dietary administration of formulated fenaminosulf was not carcinogenic in either Fischer 344 rats or B6C3F$_1$ mice.

Synonyms: sodium 4-(dimethylamino)phenol diazenesulfonate; p-dimethylaminobenzenediazo sodium sulfonate; sodium 4-dimethylaminobenzenediazosulfonate; DAS; Dexon®; Diazoben; Bayer 22555

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-102 Bioassay of 3-Sulfolene for Possible Carcinogenicity (CAS No. 77-79-2)

3-Sulfolene is an intermediate in the production of sulfolane, which is used in the petroleum, plastics, and textile industries, and in the synthesis of one of more fungicides or additional chemicals. 3-Sulfolene is also used as a catalyst.

A bioassay of 3-sulfolene for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. 3-Sulfolene in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The 78-week period of chemical administration was followed by an observation period of 33 weeks for the high dose female rats and low dose rats of both sexes. The last high dose male rat died in week 60. All treated groups of mice were observed for an additional 13 weeks following chemical administration.

Initial dosage levels for the chronic bioassay were selected on the basis of a preliminary subchronic toxicity test. Subsequent dosage adjustments were made during the course of the chronic bioassay. The time-weighted average high and low doses of 3-sulfolene in the chronic study were respectively, 372 and 197 mg/kg/day for male rats, 240 and 120 mg/kg/day for female rats, 622 and 311 mg/kg/day for male mice and 768 and 384 mg/kg/day for the female mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil at the same time that dosed animals were gavaged with the 3-sulfolene mixtures. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

There was a significant positive association between the administered dosages of 3-sulfolene and mortality in both sexes of rats and mice. In all groups, except the high dose male rats and the high dose male and female mice, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors.

There were no tumors in either sex of rats or mice for which a significant positive association could be established between chemical administration and incidence.

Under the conditions of this bioassay, there was no evidence for the carcinogenicity of 3-sulfolene to Osborne-Mendel rats or B6C3F$_1$ mice.

Synonyms: 2,5-dihydrothiophene 1,1-dioxide; 1-thia-3-cyclopentene 1,1-dioxide; butadiene sulfone

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-103 Bioassay of Fenthion for Possible Carcinogenicity (CAS No. 55-38-9)

Fenthion, the O,O-dimethyl ester of O-(4-(methylthio)-m-tolylphosphorothioic acid, is one of the organophosphate pesticides. It was developed by G. Schrader and E. Schegk and first marketed by Farbenfabriken Bayer A.G. as an insecticide in 1957.

A bioassay of fenthion for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F$_1$ mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered fenthion in the diet at one of two doses, either 10 or 20 ppm, for 103 weeks and then observed for 0 to 2 additional weeks. Matched controls consisted of groups of 25 untreated animals of each species and sex. All surviving animals were killed at 103 to 105 weeks.

The mean body weights and the survival of the dosed animals were essentially unaffected by administration of the test chemical with the exception of the survival of the low-dose male mice, which was significantly lower than that of the corresponding matched control. Thus, most of the animals may have been able to tolerate higher doses. Sufficient numbers of animals in all groups of rats and mice were at risk for development of late-appearing tumors.

In the male and female rats and the female mice, no tumors occurred at incidences that were significantly higher in dosed groups than in control groups.

In the male mice, sarcomas, fibrosarcomas, or rhabdomyosarcomas of the integumentary system occurred at incidences that were dose related ($P = 0.043$). In direct comparisons of the incidences of these tumors in the dosed groups with the incidence in the control group, the P values of 0.048 and 0.028 for the low- and high-dose groups, respectively, did not meet the Bonferroni criterion of $P = 0.025$ for significance when multiple comparisons are made (controls 0/25, low-dose 7/49 or 14%, high-dose 8/48 or 17%). However, the incidence of sarcomas and fibrosarcomas in historical-control male B6C3F$_1$ mice used in bioassays of other chemicals tested at this laboratory was 7/435 (1.6%), and no rhabdomyosarcomas occurred in the historical-control male mice.

It is concluded that under the conditions of this bioassay, fenthion was not carcinogenic for male or female F344 rats or for female B6C3F$_1$ mice. The increased incidence of sarcomas, fibrosarcomas, and especially rhabdomyosarcomas of the integumentary system in the male B6C3F$_1$ mice suggested that the test chemical was carcinogenic in these animals.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Negative

TR-104 Bioassay of Anilazine for Possible Carcinogenicity (CAS No. 101-05-3)

Anilazine is a triazine fungicide originally synthesized and screened for herbicidal activity. Although anilazine is virtually nonphytotoxic, it was found to have broad-spectrum fungicidal effects, and was marketed in 1955 as an agricultural fungicide.

A bioassay of anilazine for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered anilazine at one of two doses, either 500 or 1,000 ppm, for 103 weeks and then observed for 1-6 additional weeks. Matched controls consisted of 25 untreated rats and 25 untreated mice of each sex. All surviving rats were killed at 103-104 weeks; all surviving mice were killed at 107-109 weeks.

Administration of the anilazine had no appreciable effect on body weight in the rats and female mice; however, there was a decreased gain in mean body weight in the dosed male mice. Survival also was essentially unaffected. Survival in all groups of dosed and control rats and mice was at least 80% at the end of 90 weeks on study, except for the male control mice; thus, sufficient numbers of animals were at risk in most groups for development of late-appearing tumors.

No tumors occurred in dosed rats or mice of either sex at incidences that were significantly higher than those in corresponding controls. Male and female rats and female mice may have been able to tolerate higher doses.

It is concluded that under the conditions of this bioassay, anilazine was not carcinogenic for either Fischer 344 rats or B6C3F$_1$ mice.

Synonym: 2,4-dichloro-6-(o-chloroanilino)-s-triazine

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-105 Bioassay of m-Cresidine for Possible Carcinogenicity (CAS No. 102-50-1)

m-Cresidine, an aromatic amine and dyestuff intermediate, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer associated with exposure to aromatic amines and several other classes of chemicals among workers in the dye manufacturing industry.

A bioassay of technical-grade m-cresidine for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F$_1$ mice. m-Cresidine in corn oil was administered by gavage five days a weeks at either of two dosages, to groups of 50 male and 49 or 50 female animals of each species. The dosages used in the chronic bioassay for low and high dose rats were 0.08 and 0.16 gm/kg/day, respectively. The time-weighted average dosages used for low and high dose mice were 0.06 and 0.11 gm/kg/day, respectively. After a 77-week dosing period observation of rats continued for an additional 32 to 33 weeks. After a 53-week dosing period, observation of mice continued for an additional observation period of up to 41 weeks. For each species, 50 animals of each sex were placed on test as untreated controls and 25 animals of each sex were placed on test as vehicle controls.

The urinary bladder and the kidney were the target organs of m-cresidine toxicity in male and female rats. Papillary transitional-cell carcinomas of the urinary bladder occurred in 0/45 low dose males, 5/44 (11 percent) high dose males, 1/46 (2 percent) low dose females, and 2/44 (5 percent) high dose females but did not occur in any untreated control or vehicle control rats. Although the incidences in each dosed rat group were not statistically significant when compared to vehicle controls, comparison with historical controls indicates that these bladder carcinomas are rare and are, therefore, considered to be compound-related in both sexes.

Among mice, dose-related nonneoplastic lesions were observed at higher incidences for males than females in the kidneys, spleen and thymus. Dose-related toxic effects were also observed in testes of male mice. No neoplasms occurred in male mice at statistically significant incidences.

Under the conditions of this bioassay, m-cresidine was carcinogenic to Fischer 344 rats, causing papillary transitional-cell carcinomas of the urinary bladder in both sexes. No convincing evidence was provided for carcinogenicity in female B6C3F$_1$ mice. Poor survival of male B6C3F$_1$ mice receiving m-cresidine precluded evaluation of the possible carcinogenicity of the compound in these animals.

Synonyms: 4-methoxy-2-methylbenzenamine; 4-methoxy-2-methylaniline; 2-methyl-p-anisidine

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Inadequate Study
Female Mice:	Negative

TR-106 Bioassay of Trichlorofluoromethane for Possible Carcinogenicity (CAS No. 75-69-4)

Trichlorofluoromethane, a widely used halocarbon aerosol propellant and refrigerant, was selected for bioassay by the National Cancer Institute because of widespread exposure to this compound resulting from the indiscriminate use of aerosol sprays, and the well-documented hepatocarcinogenicity of the structurally analogous compound, carbon tetrachloride.

The bioassay of technical-grade trichlorofluoromethane for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. Trichlorofluoromethane in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species, 5 days per week, over a period of 78 weeks. The time-weighted average high and low dosages of trichlorofluoromethane in the chronic bioassay were, respectively, 977 and 488 mg/kg/day for male rats, 1,077 and 538 mg/kg/day for female rats, and 3,925 and 1,962 mg/kg/day for mice of both sexes. After a 78-week dosing period, rats were observed for an additional period of up to 33 weeks and mice were observed for an additional period of up to 13 weeks.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil at the same time that dosed animals were gavaged with trichlorofluoromethane. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not gavaged.

A high rate of early deaths occurred among male and female rats in this bioassay. An insufficient number of rats of either sex survived long enough to be at risk from late- developing tumors. Survival of mice was adequate for meaningful statistical analysis of late-developing tumors.

Results of a time-adjusted statistical analysis of tumor incidence in rats indicated no significant positive associations between administration of trichlorofluoromethane and tumor incidence.

No groups of male or female mice dosed with trichlorofluoromethane had significantly increased tumor incidences relative to their respective control groups.

The results of the bioassay of trichlorofluoromethane in Osborne-Mendel rats for possible carcinogenicity are not conclusive because inadequate numbers of rats survived sufficiently long enough to be at risk from late-developing tumors. Under the conditions of this bioassay, trichlorofluoromethane was not carcinogenic to male or female B6C3F$_1$ mice.

Synonyms: trichloromonofluoromethane; fluorotrichloromethane; Fluorocarbon 11; Propellant 11; Freon 11®; Arcton 11®; Frigen 9®

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Inadequate Study
Female Rats:	Inadequate Study
Male Mice:	Negative
Female Mice:	Negative

TR-107 Bioassay of 5-Nitro-o-toluidine for Possible Carcinogenicity (CAS No. 99-55-8)

5-Nitro-o-toluidine, an intermediate in the synthesis of azo dyes, was selected for bioassay by the National Cancer Institute in an attempt to elucidate those chemicals which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry.

A bioassay of 5-nitro-o-toluidine for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. 5-Nitro-o-toluidine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The time-weighted average high and low dietary concentrations of 5-nitro-o-toluidine were, respectively, 0.01 and 0.005 percent for rats, and 0.23 and 0.12 percent for mice. After a 78-week period of compound administration, observation of the rats continued for an additional 30 to 31 weeks and observation of the mice continued for up to an additional 20 weeks.

For each species, 50 animals of each sex were placed on test as controls and fed only the basal diet.

There were no significant positive associations between the concentration of 5-nitro-o-toluidine administered and mortality in rats or mice of either sex, and adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

No unusual tumors were observed in rats of either sex and no convincing statistical evidence was provided for a significant positive association between compound administration and the incidence of any neoplasm in rats.

Among mice there was a statistically significant positive association between administration of the chemical and the incidences of hepatocellular carcinomas in both males and females. The combined incidence of hemangiomas and hemangiosarcomas in male mice and the incidence of hemangiosarcomas in female mice were not statistically significant in relation to their respective control groups. However, they were considered to be associated with compound administration since they rarely occur in untreated $B6C3F_1$ mice.

Under the conditions of this bioassay 5-nitro-o-toluidine was carcinogenic in $B6C3F_1$ mice, causing hepatocellular carcinomas in both sexes, an increase in the combined incidence of hemangiomas and hemangiosarcomas in male mice, and an increased incidence of hemangiosarcomas in female mice. The compound was not carcinogenic in Fischer 344 rats.

Synonyms: 2-methyl-5-nitro-benzeneamine; 4-nitro-2-aminotoluene; 6-methyl-3-nitroaniline; Fast Scarlet Base G; Colour Index Azoic Diazo Component 12

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-108 C.I. Direct Black 38 (CAS: 1937-37-7) C.I. Direct Blue 6 (CAS: 2602-46-2) C.I. Direct Brown 95 (CAS: 16071-86-6)

These studies were reported in the NCI Carcinogenesis Technical Report Series and assigned a Technical Report number; however because they reflect the results of 13-week subchronic studies, abstracts are not included in this compendium. See *Appendix A, Table A1* for ordering information.

TR-109 Bioassay of 4-Nitroanthranilic Acid for Possible Carcinogenicity (CAS No. 619-17-0)

4-Nitroanthranilic acid, a nitrobenzene derivative formerly used as a dye intermediate, was selected for bioassay by the National Cancer Institute along with other dye intermediates in an attempt to identify those chemicals which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry.

A bioassay of 4-nitroanthranilic acid for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. 4-Nitroanthranilic acid was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low time-weighted average concentrations used for the chronic study were, respectively, 1.5 and 0.46 percent for rats and 1.0 and 0.46 percent for mice. After a 78-week period of chemical administration, the rats were observed for an additional period of up to 32 weeks and the mice for an additional period of up to 17 weeks. For rats 50 animals of each sex were placed on test as low dose controls and 25 animals of each sex were placed on test as high dose controls. For mice 50 animals of each sex were placed on test as controls for each dose group.

No statistically significant increases in tumor incidence were observed among rats or mice receiving diets containing 4-nitroanthranilic acid.

Under the conditions of this bioassay evidence was not provided for the carcinogenicity of 4-nitroanthranilic acid in Fischer 344 rats or B6C3F$_1$ mice.

Synonym: 2-amino-4-nitro-benzoic acid

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-110 Bioassay of Iodoform for Possible Carcinogenicity (CAS No. 75-47-8)

Iodoform, a halogenated alkane with antiseptic and anti-infective properties, was selected for bioassay by the National Cancer Institute because of its similarity to methyl iodide, which has produced sarcomas in BD rats and to chloroform, a compound which has been found to induce hepatomas in NLC mice.

A bioassay for possible carcinogenicity of technical-grade iodoform was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. Iodoform in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. Administration of the chemical occurred 5 days per week, for a period of 78 weeks, followed by an observation period of 34 weeks for rats and 13 or 14 weeks for mice. The high and low time-weighted average dosages of iodoform were, respectively, 142 and 71 mg/kg/day for male rats, 55 and 27 mg/kg/day for female rats, and 93 and 47 mg/kg/day for male and female mice. For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with pure corn oil at the same rate as the high dose group of the same sex. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not intubated.

A significant positive association between dosage and mortality was observed in male rats but not in female rats or in mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

No statistical significance could be attributed to the incidences of any neoplasms in rats or mice of either sex when compared to their respective controls.

Under the conditions of this bioassay, no convincing evidence was provided for the carcinogenicity of iodoform in Osborne-Mendel rats or B6C3F$_1$ mice.

Synonym: triiodo-methane

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-111 Bioassay of 1-Amino-2-methylanthraquinone for Possible Carcinogenicity (CAS No. 82-28-0)

1-Amino-2-methylanthraquinone, an intermediate in the synthesis of anthraquinone dyes and a dye itself, was selected for bioassay by the National Cancer Institute in an attempt to elucidate those chemicals which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry.

A bioassay for possible carcinogenicity of technical-grade 1-amino-2-methylanthraquinone was conducted using Fischer 344 rats and B6C3F$_1$ mice. 1-Amino-2-methylanthraquinone was administered in the feed, at either of two concentrations, to groups of 45 to 50 males and females of each species. The high and low time-weighted average concentrations of 1-amino-2-methylanthraquinone were 0.20 and 0.10 percent, respectively, for male and female rats. For mice, two dosage regimens (designated A and B) were used, but the time-weighted average concentrations were the same, 0.06 percent. For each species, 50 animals of each sex were placed on test as controls. The period of compound administration was 78 weeks for rats followed by 26 to 28 additional weeks of observation, and 73 weeks for mice followed by 24 to 25 additional weeks of observation.

A statistically significant positive association between compound administration and mortality was established for the male and female dose A mice. Dose A mice did not survive sufficiently long to be at risk from late-developing tumors. Survival in all other groups was adequate.

The incidence of hepatocellular carcinomas was statistically significant among dosed rats of both sexes. Kidney neoplasms (the combined incidence of tubular-cell adenomas, tubular-cell adenocarcinomas, and adenocarcinomas NOS) were significantly increased among dosed male rats.

Administration of the compound was associated with a significant increase in the combined incidence of hepatocellular carcinomas and neoplastic liver nodules in female mice. No other neoplasms occurred in statistically significant positive incidences in male or female mice. 1-Amino-2-methylanthraquinone demonstrated nephrotoxic properties in mice of both sexes.

Under the conditions of this bioassay, 1-amino-2-methylanthraquinone was carcinogenic in Fischer 344 rats, inducing hepatocellular carcinomas in rats of both sexes, and kidney tumors in male rats. The compound was carcinogenic in female B6C3F$_1$ mice, producing an increased combined incidence of hepatocellular carcinomas and neoplastic nodules.

Synonyms: 1-amino-2-methyl-9,10-anthracenedione; 2-methyl-1-anthraquinonylamine; Disperse Orange 11

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Positive

TR-112 Bioassay of 3-Amino-4-ethoxyacetanilide for Possible Carcinogenicity (CAS No. 17026-81-2)

3-Amino-4-ethoxyacetanilide, an azo dye intermediate closely related to the para-aminophenol analgesics, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer reported among workers in the dye manufacturing industry.

A bioassay of 3-amino-4-ethoxyacetanilide for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F$_1$ mice. 3-Amino-4-ethoxyacetanilide was administered in the feed, at either of two concentrations, to groups of approximately 50 male and 50 female animals of each species. The dietary concentrations used in the chronic bioassay for low and high dose rats were 0.4 and 1.5 percent, respectively. The dietary concentrations used for low and high dose mice were 0.4 and 0.8 percent, respectively. After a 78-week period of chemical administration, observation of rats continued for up to 18 weeks. For each species, 50 animals of each sex were placed on test as controls for low dose groups and approximately 50 animals of each sex were placed on test as controls for high dose groups.

In both species, adequate numbers of animals in all groups survived sufficiently long enough to be at risk from late-developing tumors.

Among rats the only clearly compound-related lesion was hemosiderosis of the thyroid gland. No neoplastic lesions were statistically significant in dosed rats.

Among mice the incidence of follicular-cell carcinomas of the thyroid gland was significant for high dose males. An elevated incidence of thyroid hyperplasia was observed in each dosed group. Hemosiderosis of the thyroid cells were found in nearly all dosed mice, but not in any control mice.

Under the conditions of this bioassay, 3-amino-4-ethoxyacetanilide was carcinogenic in male B6C3F$_1$ mice, causing follicular-cell carcinomas of the thyroid gland. Evidence provided by this bioassay was insufficient to establish the carcinogenicity of 3-amino-4-ethoxyacetanilide in female mice or in Fischer 344 rats of either sex.

Synonyms: 3-amino-4-ethoxy-N-phenylacetamide; N-(3-amino-4-ethoxyphenyl) acetamide; 3-amino-p-acetophenetidin

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Negative

TR-113 Bioassay of 2-Chloro-p-phenylenediamine Sulfate for Possible Carcinogenicity (CAS No. 61702-44-1)

2-Chloro-p-phenylenediamine sulfate is a salt of 2-chloro-p-phenylenediamine and sulfuric acid. It is a component of commercial hair dyes and was selected for bioassay by the National Cancer Institute because of the increased bladder cancer incidence noted among dye manufacturing workers.

A bioassay for possible carcinogenicity of 2-chloro-p-phenylenediamine sulfate was conducted using Fischer 344 rats and B6C3F$_1$ mice. 2-Chloro-p-phenylenediamine sulfate was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The dietary concentrations used in the chronic bioassay were 0.3 and 0.15 percent for the high and low dose rats, respectively, and 0.6 and 0.3 percent for the high and low dose mice, respectively. Compound administration was for 105 to 107 weeks in rats, 87 weeks in high dose mice, and 104 to 105 weeks in low dose mice. The only groups observed during an untreated period after dosing were the high dose mice, observed for 18 weeks after compound administration ceased. For each species, 20 animals of each sex were placed on test as controls.

There were no significant positive associations between the administered dietary concentrations of 2-chloro-p-phenylenediamine sulfate and mortality for rats of either sex or male mice. There was a significant positive association between dosage and mortality for female mice; however, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

There were no statistically significant positive associations between dietary exposure to the compound and the incidences of any tumor in rats. There was an increased incidence of transitional-cell hyperplasia of the renal pelvic epithelium in both male and female rats, and transitional-cell tumors of the urinary bladder were present in three dosed rats. These lesions indicated a possible carcinogenic effect, but are not considered as sufficient evidence of carcinogenicity. In mice, no tumors occurred in statistically significantly higher incidences in the dosed mice than in controls.

Under the conditions of this bioassay there was insufficient evidence that dietary administration of 2-chloro-p-phenylenediamine sulfate was carcinogenic to Fischer 344 rats and B6C3F$_1$ mice.

Synonyms: 2-chloro-1,4-benzenediamine sulfate; 3-chloro-4-aminoaniline sulfate; o-chloro-p-phenylenediamine sulfate; C.I. Oxidation Base 13A; Ursol Brown 0

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-114 Bioassay of 2,3,5,6-Tetrachloro-4-nitroanisole for Possible Carcinogenicity (CAS No. 2438-88-2)

2,3,5,6-Tetrachloro-4-nitroanisole, an agricultural fungicide and acaricide, was selected for bioassay by the National Cancer Institute because of its structural similarity to pentachloronitrobenzene, a pesticide classified as tumorigenic by the Secretary's Commission on Pesticides and their Relationship to Environmental Health.

A bioassay for possible carcinogenicity of 2,4,5,6-tetrachloro-4-nitroanisole was conducted using Fischer 344 rats and B6C3F$_1$ mice. 2,3,5,6-Tetrachloro-4-nitroanisole was administered in the feed, at either of two concentrations, to groups of male and female animals of each species. The high and low dietary concentrations used in the chronic bioassay were 0.012 and 0.006 percent, respectively, for both species. After a 104-week period of chemical administration, observation of rats continued for up to 3 weeks and observation of mice continued for up to 1 week. For rats 50 animals of each sex were placed on test as controls, while for mice 55 animals of each sex were placed on test as controls.

There were no significant positive associations between the dietary concentration of 2,3,5,6-tetrachloro-4-nitroanisole administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

No neoplasms, except for interstitial-cell testicular tumors in males, occurred at statistically significant incidences

Synonyms: 1-methoxy-4-nitro-2,3,5,6-tetrachlorobenzene; tetrachloronitroanisole; ENT 33225; TCNA

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-115 Bioassay of Sulfallate for Possible Carcinogenicity (CAS No. 95-06-7)

Sulfallate, a chlorinated dithiocarbamate derivative used as a preemergence herbicide on vegetable crops, was selected for bioassay by the National Cancer Institute because of its structural relationship to the known tumorigens selenium diethyldithiocarbamate and potassium bis(2-hydroxyethyl) dithiocarbamate and to a number of suspected tumorigens containing the diethyldithiocarbamate or dimethyldithiocarbamate moieties.

A bioassay for possible carcinogenicity of sulfallate was conducted using Osborne-Mendel rats and B6C3F$_1$ mice. Sulfallate was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty mice and 50 rats of each sex were placed on test as controls for the bioassay. The time-weighted average high and low dietary concentrations of sulfallate were, respectively, 410 and 250 ppm for male rats, 404 and 250 ppm for female rats, 1,897 and 949 ppm for male mice, and 1,815 and 908 ppm for female mice. After a 78-week period of chemical administration, there was an additional observation period of 25 to 26 weeks for dosed rats, 33 weeks for control rats, and 12 to 13 weeks for dosed and control mice.

There were significant positive associations between increased sulfallate concentration and accelerated mortality in both sexes of rats and in female mice. However, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

Statistical analyses of the incidences of mammary adenocarcinomas in female rats, stomach neoplasms (i.e., combination of papillomas NOS, squamous-cell papillomas, and squamous-cell carcinomas) in male rats, combined alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas in male mice, and adenocarcinomas of the mammary gland in female mice revealed a significant positive association between dosage and incidence. These associations were all supported by at least one significant Fisher exact comparison.

The incidences of toxic tubular nephropathy observed in male rats and in mice of both sexes increased with the concentration of the compound administered. This nonneoplastic lesion was not observed in control animals.

Under the conditions of this bioassay, dietary administration of sulfallate was carcinogenic to Osborne-Mendel rats and to B6C3F$_1$ mice, inducing mammary gland tumors in females of both species, tumors of the forestomach in male rats, and lung tumors in male mice.

Synonyms: diethylcarbamodithioic acid 2-chloro-2-propenyl ester; 2-chloro-2-propenyl diethylcarbamodithioate; 2-chloroallyl diethyldithiocarbamate; diethyldithiocarbamic acid 2-chloroallyl ester; CDEC; CP4742

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-116 Bioassay of p-Anisidine Hydrochloride for Possible Carcinogenicity (CAS No. 20265-97-8)

p-Anisidine hydrochloride, the hydrochloride salt of an aromatic dye intermediate, was selected for bioassay by the National Cancer Institute because of the increased bladder cancer incidence noted among workers in the dye manufacturing industry.

A bioassay for possible carcinogenicity of p-anisidine hydrochloride was conducted using Fischer 344 rats and $B6C3F_1$ mice. p-Anisidine hydrochloride was administered in the feed, at either of two concentrations, to groups of 55 male and 55 female animals of each species. Fifty-five animals of each sex and species were placed on test as controls. The high and low dietary concentrations of p-anisidine hydrochloride were, respectively, 0.6 and 0.3 percent for rats and 1.0 and 0.5 percent for mice. The compound was administered in the diet for 103 weeks, followed by an observation period of 2 to 3 weeks for rats and 2 weeks for mice.

There were no significant positive associations for either species between the concentration of p-anisidine hydrochloride administered and mortality. In addition, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

In male rats there were significant associations between compound administration and the incidences of both squamous-cell carcinomas of the skin and alveolar/bronchiolar adenomas. None of the Fischer exact comparisons, however, supported these findings. When those males having adenomas NOS or carcinomas NOS of the preputial gland were combined and the resulting incidences statistically analyzed, the only test providing a significant result was the Fisher exact comparison of the low dose to the control. There were no significant positive associations between the administration of p-anisidine hydrochloride and the incidence of any tumor in mice of either sex.

Although, under the conditions of this bioassay, there appeared to be an association between chemical administration and the increased incidence of preputial gland tumors in low dose male rats, the evidence was insufficient to establish the carcinogenicity of p-anisidine hydrochloride in Fischer 344 rats. The compound was not carcinogenic in $B6C3F_1$ mice.

Synonyms: 4-methoxy-benzeneamine HCl; p-aminoanisole HCl; 4-methoxyaniline HCl

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-117 Bioassay of 6-Nitrobenzimidazole for Possible Carcinogenicity (CAS No. 94-52-0)

6-Nitrobenzimidazole, a heterocyclic aromatic compound used in photographic developers, was selected for bioassay by the National Cancer Institute because of the suspect status of aromatic nitro- compounds.

A bioassay for possible carcinogenicity of 6-nitrobenzimidazole was conducted using Fischer 344 rats and $B6C3F_1$ mice. 6-Nitrobenzimidazole was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The dietary concentrations used in the chronic bioassay were 0.5 and 0.12 percent for the high and low dose rats, respectively, and 0.24 and 0.12 percent for the high and low dose mice, respectively. After a 78-week period of compound administration, observation of the rats continued for up to an additional 29 weeks and observation of the mice continued for an additional 18 weeks. For each species and each dosed group, 49 or 50 animals of each sex were placed on test as controls.

There were no significant positive associations between the administered dietary concentrations of 6-nitrobenzimidazole and mortality in either sex of rats or mice. In all groups adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors.

Among both male and female mice, the incidences of hepatocellular carcinomas in high dose groups were statistically significant relative to controls.

Among rats of both sexes, nonneoplastic lesions of the eyes and of the Harderian glands appeared to be associated with administration of 6-nitrobenzimidazole. No neoplasms, however, were attributed to compound administration.

Under the conditions of this bioassay, dietary administration of 6-nitrobenzimidazole was not carcinogenic to Fischer 344 rats; however, the compound was carcinogenic to $B6C3F_1$ mice, causing hepatocellular carcinomas in both sexes.

Synonym: 5-nitro-1H-benzimidazole

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-118 Bioassay of 5-Nitroacenaphthene for Possible Carcinogenicity (CAS No. 602-87-9)

5-Nitroacenaphthene has never had any known commercial application in the United States and is apparently produced in this country solely for research purposes. The compound is, however, used in Japan as a captive

intermediate in the synthesis of naphthalimide dyes; 95 percent of these dyes find application as fluorescent whitening agents in laundry detergents while the remainder are used to dye paper.

A bioassay of 5-nitroacenaphthene for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F$_1$ mice. 5-Nitroacenaphthene was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. For male and female rats, the high and low dietary concentrations of 5-nitroacenaphthene were 0.24 and 0.12 percent, respectively. The high and low time-weighted average concentrations for mice were 0.12 and 0.06 percent, respectively, for males and 0.12 and 0.05 percent, respectively, for females. After a 78-week dosing period, observation of surviving rats continued for up to 22 weeks and observation of the mice continued for 18 weeks.

For the chronic rat bioassay, 49 male and 50 female rats were placed on test as high dose controls, and 50 rats of each sex served as low dose controls. For the mice, 50 males and 50 females were placed on test as controls.

Accelerated mortality was observed in all dosed groups except the low dose female mice. There was a positive association between mortality and dietary concentration of 5-nitroacenaphthene for both sexes of both species. Early deaths were most apparent among high dose male mice; half of the animals in this group were dead by week 20 and insufficient male mice survived to be at risk from late-developing tumors.

Among rats, the incidence of malignant tumors of the ear canal (incidences of ceruminous carcinomas and squamous-cell carcinomas were combined) was significant at each dose level in each sex. Among both dosed groups of female rats, the incidence of clitoral gland carcinoma and the incidence of mammary adenocarcinoma were significant. A significant incidence of alveolar/bronchiolar carcinoma was observed in low dose rat groups of each sex.

Among female mice, the incidence of hepatocellular carcinoma was significant at each dose level. The combined incidence of granulosa-cell tumors, luteomas, and tubular-cell adenomas of the ovary was significant in the high dose female mouse group.

Under the conditions of this bioassay, 5-nitroacenaphthene was carcinogenic to Fischer 344 rats, causing increased incidences of malignant tumors of the ear canal and lung in both sexes, and of the clitoral gland and mammary gland in females. 5-Nitroacenaphthene was also carcinogenic to female but not male B6C3F$_1$, mice, causing carcinomas of the liver and ovarian tumors.

Synonyms: 1,2-dihydro-5-nitro-acenaphthylene; 5-nitro-acenaphthylene

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Positive

TR-119 Carbon Disulfide (CAS: 75-15-0)

Data considered to be inconclusive and not reportable; no Technical Report issued

TR-120 Bioassay of Piperonyl Butoxide for Possible Carcinogenicity (CAS No. 51-03-6)

Piperonyl butoxide is used to enhance the insecticidal properties of the pyrethrins by blocking the pyrethrin detoxification enzymes in the insect. Pyrethrins alone produce a very rapid knockdown of insects followed by substantial recovery, whereas addition of a synergist such as piperonyl butoxide decreases the insecticidal dose of pyrethrin. Piperonyl butoxide is also formulated with synthetic pyrethrin analogues, such as allethrin and tetramethrin.

A bioassay of technical-grade piperonyl butoxide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 50 rats of each sex were administered piperonyl butoxide in the diet at one of two doses, either 5,000 or 10,000 ppm, for 107 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at the end of the period of administration of the test chemical.

Groups of 50 mice of each sex were initially administered piperonyl butoxide at one of two doses, either 2,500 or 5,000 ppm. After week 30, the doses for the mice were reduced to 500 and 2,000 ppm, respectively, and administration of the test chemical at the lowered doses was continued for 82 weeks. The time-weighted average doses for the mice were either 1,036 or 2,804 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of the period of administration of the test chemical.

Mean body weights of dosed groups of rats and mice of each sex were lower than those of corresponding control groups, and the depressions in body weights were dose related. Survival of the rats and mice was unaffected by the piperonyl butoxide and was 80% or greater in all groups at week 90 of the bioassay; thus, sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors.

In female rats, lymphomas occurred at incidences that were dose related ($P = 0.007$); in a direct comparison, the incidence of the tumor in the high-dose group was higher ($P = 0.020$) than that in the control group (controls 1/20, low-dose 7/50, high-dose 15/50). However, the incidence of lymphomas, leukemias, and reticuloses in historical-control female Fischer 344 rats at the same laboratory was 19/191 (10%). These historical-control groups include one with an incidence of animals with lymphoma or leukemia of 7/20 (35%) and another with an incidence of 6/20 (30%). Thus, the incidence of lymphomas in the control female rats of the present bioassay may have been

abnormally low, and the occurrence of the higher incidence in the dosed groups cannot be clearly related to administration of piperonyl butoxide.

In the male mice, adenomas of the lacrimal gland occurred at incidences that were dose related (P = 0.023), but in direct comparisons the incidences in the individual dosed groups were not significantly higher than that in the control group (controls 0/20, low-dose 0/49, high-dose 4/50); thus, the occurrence of this tumor in the male mice was not clearly related to administration of the test chemical.

It is concluded that under the conditions of this bioassay, piperonyl butoxide was not carcinogenic for Fischer 344 rats or B6C3F$_1$ mice.

Synonym: 5-((2-(2-butoxyethoxy)ethoxy)-methyl-6-propyl-1,3-benzodioxole

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-121 Bioassay of Dimethyl Terephthalate for Possible Carcinogenicity (CAS No. 120-61-6)

Dimethyl terephthalate is one of the basic monomers used in the synthesis of polyester fibers.

A bioassay of dimethyl terephthalate for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F$_1$ mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered dimethyl terephthalate at one of two doses, either 2,500 or 5,000 ppm, for 103 weeks, then observed for 2 additional weeks. Matched controls consisted of 50 untreated rats of each sex and 50 untreated mice of each sex. All surviving rats were killed at 105 or 106 weeks and all surviving mice at 104 or 105 weeks.

Administration of dimethyl terephthalate had no appreciable effect on the mean body weights of the rats and mice of either sex. No clinical signs related to administration of the test chemical were noted in the rats. Survivals of the rats and mice at the end of the bioassay were not affected by the test chemical. Both species may have been able to tolerate higher doses.

In rats and mice of each sex, no tumors occurred at incidences that clearly were related to administration of the test chemical.

Although it is recognized that both rats and mice may not have received a dose of the test chemical sufficiently high to provide maximum test sensitivity, it is concluded that under the conditions of this bioassay, dimethyl terephthalate was not carcinogenic for F344 rats or B6C3F$_1$ mice.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Negative

TR-122 Bioassay of Dibenzo-p-dioxin for Possible Carcinogenicity (CAS No. 262-12-4)

Unsubstituted dibenzo-p-dioxin (UDD), is an analog of a series of chlorinated dibenzo-p-dioxins that were selected for carcinogenesis testing. The chlorinated compounds are formed as unwanted by-products during the synthesis of chlorophenols, and were discovered in the late 1960's as contaminants in the industrial microbicide pentachlorophenol, and in a widely used agricultural herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

A bioassay of UDD for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and B6C3F$_1$ mice.

Groups of 35 rats of each sex were administered UDD at one of two doses, either 5,000 or 10,000 ppm, for 110 weeks. Groups of 50 mice of each sex were administered the same doses for 87 or 90 weeks. Controls consisted of groups of 35 untreated rats of each sex and 50 untreated mice of each sex. All surviving male rats were killed at 110 weeks, all surviving male mice at 92 to 97 weeks, and all surviving female mice at 91 to 93 weeks.

Mean body weights of the dosed male and female rats and mice were lower than those of the corresponding controls; the depression in the amount of weight gained in the dosed male mice was, however, relatively slight. Except for the male rats, survival at the end of the bioassay was lower in the dosed groups of both rats or mice than in the corresponding control groups. At week 90, at least 57% of the rats and 54% of the mice were still alive. Because the mean body weights and survival rates of the dosed animals were lower than those of corresponding controls and because there was an increase in the incidence of hepatotoxic lesions, the 10,000-ppm concentration administered to the rats and mice is considered to be the maximum tolerated dose.

No tumors were induced in rats or mice of either sex at incidences that were significantly higher in the dosed groups than in the corresponding control groups.

It is concluded that under the conditions of this bioassay, UDD was not carcinogenic for Osborne-Mendel rats or B6C3F$_1$ mice.

Synonym: UDD

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-123 Bioassay of 2,7-Dichlorodibenzo-p-dioxin (DCDD) for Possible Carcinogenicity (CAS No. 33857-26-0)

2,7-Dichlorodibenzo-p-dioxin, referred to in this report as DCDD, is a chlorinated dibenzodioxin. Chlorinated dibenzodioxins have been found as by-products in the manufacture of pentachlorophenol and in the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its esters. Pentachlorophenol is a microbicidal agent that is used as a wood preservative, for slime control in herbicide formulations, and in the manufacture of paper pulp; 2,4,5-T has been used as a herbicide on national forests, rangelands, pastures, in the agricultural industry, and as a component of Agent Orange, a wartime defoliant.

A bioassay of DCDD for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and $B6C3F_1$ mice.

Groups of 35 rats of each sex were administered DCDD at one of two doses, either 5,000 or 10,000 ppm, for 110 weeks. Groups of 50 mice of each sex were administered these same doses for 90 weeks. Controls consisted of 35 untreated rats of each sex and 50 untreated mice of each sex. All surviving male rats were killed at 110 to 112 weeks, all surviving female rats at 110 to 117 weeks, all surviving male mice at 92 to 101 weeks, and all surviving female mice at 91 to 98 weeks.

Mean body weights of most of the dosed groups of rats and mice were lower than those of corresponding controls both when placed on study and for much of the study period; however, survival of any group was not significantly affected by administration of the test chemical. Sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors.

No tumors were induced in male or female rats or female mice at incidences that were significantly higher in the dosed groups than in the corresponding control groups. Both low- and high-dose rats had toxic hepatic lesions characterized by centrilobular fatty metamorphosis and/or necrosis.

In the male mice, hepatocellular adenomas or carcinomas occurred at incidences that were dose related ($P = 0.008$), and, in direct comparisons, were higher in the low-dose group ($P = 0.008$) and the high-dose group ($P = 0.010$) than in the control group (controls 8/49, low-dose 20/50, high-dose 17/42). However, the historical incidence of this lesion in control male $B6C3F_1$ mice at this laboratory does not permit a clear association of the lesion with the administration of the test compound. There were also significant increases in the incidence of combinations of leukemias and lymphomas and of hemangiosarcomas and hemangiomas in the low-dose male mice, but these findings were not supported by the high-dose animals.

It is concluded that under the conditions of this bioassay, DCDD was not carcinogenic for Osborne-Mendel rats of either sex or for female $B6C3F_1$ mice. The marginal increased incidences of combinations of leukemias and lymphomas, of hemangiosarcomas and hemangiomas, and of hepatocellular carcinomas and adenomas in male $B6C3F_1$ mice are, however, considered as suggestive of a carcinogenic effect of 2,7-dichlorodibenzo-p-dioxin in these animals.

Synonym: DCDD

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Negative

TR-124 Bioassay of Piperonyl Sulfoxide for Possible Carcinogenicity (CAS No. 120-62-7)

Piperonyl sulfoxide is used to enhance the insecticidal properties of the pyrethrins by inhibiting pyrethrin detoxification enzymes, probably microsomal oxidases, in the insect. Pyrethrins alone produce a transient paralysis, whereas pyrethrins combined with a synergist such as piperonyl sulfoxide are insecticidal.

A bioassay of technical-grade piperonyl sulfoxide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered piperonyl sulfoxide in the diet at one of several doses, either 1,500 or 3,000 ppm for the males and either 3,000 or 6,000 ppm for the females, for 105 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at the end of the period of administration of the test chemical.

Groups of 50 male mice were administered one of two doses, either 350 or 700 ppm, for 104 or 105 weeks. Groups of 50 female mice were initially administered one of two doses, either 700 or 1,400 ppm. Due to excessive weight depression in the dosed female mice, the doses for this sex were reduced after week 20 to 200 and 600 ppm, respectively, and administration of the test chemical at the lower doses was continued for 84 or 85 weeks. The time-weighted average doses for the females were 295 and 754 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of the period of administration of the test chemical.

Mean body weights of dosed groups of rats and mice of each sex were lower than those of corresponding control groups, and the depressions in the amount of mean body weight gained were dose related for most or all of the bioassay; the depression in the amount of mean body weight gained was slight, however, in the dosed male rats. Survival of the rats and mice was unaffected by the piperonyl sulfoxide and was 78% or higher in all groups at week 90 of the bioassay; thus sufficient numbers of dosed

and control rats and mice of each sex were at risk for the development of late-appearing tumors.

In the male and female rats and in the female mice, no tumors occurred at incidences that were significantly higher in dosed groups than in control groups.

In the male mice, hepatocellular carcinomas occurred at incidences that were dose related ($P<0.001$); in direct comparisons, the incidence of these tumors in the high-dose group was significantly higher ($P<0.001$) than that in the control group (controls 6/18, low-dose 31/50, high-dose 46/50).

It is concluded that under the conditions of this bioassay, technical-grade piperonyl sulfoxide was not carcinogenic for male or female Fischer 344 rats or for female $B6C3F_1$ mice, but was carcinogenic for male $B6C3F_1$ mice, producing an increased incidence of hepatocellular carcinomas.

Synonym: 1,2-(Methylenedioxy)-4-(2-(octylsulfinyl) propyl) benzene.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Negative

TR-125 Bioassay of Dioxathion for Possible Carcinogenicity (CAS No. 78-34-2)

Dioxathion, an organophosphorous insecticide, was selected for bioassay by the National Cancer Institute because of its widespread use on livestock and edible crops, and a lack of adequate chronic toxicity data.

A bioassay for possible carcinogenicity of technical-grade dioxathion was conducted using Osborne-Mendel and $B6C3F_1$ mice. Dioxathion was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low time-weighted average concentrations were, respectively, 180 and 90 ppm for male rats and 90 and 45 ppm for female rats. The high and low time-weighted average concentrations for male mice were 567 and 284 ppm, respectively, and for female mice were 935 and 467 ppm, respectively. After a 78-week period of chemical administration, observation of the rats continued for an additional 33 weeks and the mice were observed for an additional 12 to 13 weeks. For rats, 50 animals of each sex were placed on test as controls and fed only the basal diet, while for mice, 20 animals of each sex served as controls.

In both species adequate numbers of animals survived long enough to be at risk from late-appearing tumors.

A variety of neoplasms was observed in treated animals of both species; however, none of the neoplasms observed were either histopathologically unusual or in statistically significant incidences.

Under the conditions of this bioassay, dietary administration of dioxathion was not carcinogenic in Osborne-Mendel rats or $B6C3F_1$ mice.

Synonyms: phosphorodithioic acid, S,S'-p-dioxane-2,3-diyl O,O,O',O'-tetraethyl ester; 2,3-p-dioxanedithiol S,S-bis(O,O-diethyl phosphorodithioate; phosphorodithioic acid, S,S'-1,4-dioxane-2,3-diyl O,O,O',O'-tetraethyl ester

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-126 Bioassay of 2,5-Toluenediamine Sulfate for Possible Carcinogenicity (CAS No. 6369-59-1)

2,5-Toluenediamine sulfate, a salt of 2,5-toluenediamine and sulfuric acid, was selected for bioassay by the National Cancer Institute in an attempt to determine those dye intermediates which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry. Aromatic amines are one of several classes of chemicals thought to contribute to the increased cancer risk in this industry.

A bioassay for possible carcinogenicity of 2,5-toluenediamine sulfate was conducted using Fischer 344 rats and $B6C3F_1$ mice. 2,5-Toluenediamine sulfate was administered in the feed, at either of two concentrations, to groups of 50 males and 50 females of each species. The high and low time-weighted average concentrations of the compound were, respectively, 0.2 and 0.06 percent for rats and 0.1 and 0.6 percent for mice. Because compound administration to the high and low dose groups of each species was not begun simultaneously, each dosed group was assigned a control group. All control groups consisted of 50 animals, except for the high dose male and female rat control groups which were composed of 25 animals. The dosing period was for 78 weeks, followed by an additional 28 to 31 weeks of observation in rats and an additional 16 to 19 weeks in mice.

There was no significant association between compound administration and accelerated mortality in either sex of either species. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

A statistically significant incidence of interstitial-cell neoplasms of the testis in dosed male rats was not considered attributable to administration of the compound since the spontaneous incidence of these neoplasms in male Fischer 344 rats is both high and variable. No neoplasms were observed in female rats at statistically significant incidences.

A statistically significant increase in lung tumors in high dose female mice was not considered convincing evidence of a compound-related carcinogenic effect be-

cause high dose male mice were received in separate shipments from their controls and housed in separate rooms from their controls.

Under the conditions of this bioassay, sufficient evidence was not obtained to demostrate the carcinogenicity og 2,5-toluenediamine sulfate in either Fischer 344 rats or $B6C3F_1$ mice.

Synonyms: 2-methyl-1,4-benzenediamine sulfate; p-tolylenediamine sulfate; p-diaminotoluene sulfate; Fouramine standard; C.I. Oxidation base 4

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-127 Bioassay of 5-Nitro-o-anisidine for Possible Carcinogenicity (CAS No. 99-59-2)

5-Nitro-o-anisidine, a tri-substituted benzene derivative used as an intermediate in the synthesis of dyes, was selected for bioassay by the National Cancer Institute along with other dye intermediates in an attempt to determine which chemicals may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry. Aromatic amines and amino compounds are throught to contribute to the increased cancer risk in this industry.

A bioassay of 5-nitro-o-anisidine for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. 5-Nitro-o-anisidine was administered in the feed at either of two concentrations, to groups of 50 male and 50 female animals of each species. The dietary concentrations used in the chronic bioassay for low and high dose rats were 0.4 and 0.8 percent, respectively. Dose A and B mice were fed dietary concentrations of 0.8 and 1.6 percent when initially placed on test, but after week 15 the concentration fed to dose B mice was reduced to 0.4 percent. After a 78-week period of chemical administration, observation of rats continued for up to an additional 28 weeks and observation of mice continued for up to an additional 19 weeks. For each species, 50 animals of each sex were placed on test as controls for the group receiving the higher concentration and 49 to 50 animals were of each sex were placed on test as controls for the group receiving the lower concentration.

In both species, adequate numbers of animals in all groups survived long enough to be at risk from late-developing tumors.

Feeding of 5-nitro-o-anisidine to rats was associated with increased incidences of tumors of the integumentary system. Basal-cell carcinomas, trichoepitheliomas, squamous-cell carcinomas and sebaceous adenocarcinomas each occurred in the skin of high dose male rats at statistically significant incidences. For both male and female rats, carcinomas (the combined incidence of sebaceous adenocarcinomas, ceruminous carcinomas and squamous-cell carcinomas) of the Zymbal's gland or the skin of the ear were significant in the high dose groups. In the clitoral gland of dosed female rats, the incidence of carcinomas and the incidence of adenomas were each significant.

Among mice, the incidence of hepatocellular carcinoma was statistically significant for dose B females when compared to their appropriate controls.

Under the conditions of this bioassay, dietary administration of 5-nitro-o-anisidine was carcinogenic in Fischer 344 rats, causing tumors of the integumentary system in males and females and of the clitoral gland in females. The compound was also carcinogenic to female $B6C3F_1$ mice, causing hepatocellular carcinomas.

Synonyms: 2 - methoxy - 5 - nitro - benzeneamine; 3 - amino-4-methoxy-nitrobenzene; 2-methoxy-5-nitroaniline; 2-amino-4-nitroanisole; Fast Scarlet R; C.I. Azoic Diazo Component 13

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Equivocal
Female Mice:	Positive

TR-128 Bioassay of 3,3'-Dimethoxybenzidine-4,4'-diisocyanate for Possible Carcinogenicity (CAS No. 91-93-0)

3,3'-Dimethoxybenzidine-4,4'-diisocyanate, a dimer of o-anisidine-4,4'-diisocyanate, was selected for bioassay by the National Cancer Institute because of the structural similarity of this compound to 3,3'-dimethoxybenzidine, a carcinogen in Fischer rats.

A bioassay for the possible carcinogenicity of 3,3'-dimethoxybenzidine-4,4'-diisocyanate was conducted using Fischer 344 rats and $B6C3F_1$ mice. 3,3'-Dimethoxybenzidine-4,4'-diisocyanate was administered, at either of two concentrations, to groups of 50 male and 50 female animals of each species, with the exception of 49 high dose female rats. The compound was administered in the feed with the exception of the first 22 weeks of the rat bioassay, when it was administered by gavage. Twenty animals of each sex and species were placed on test as controls. During intubation the high and low dosages were 3,000 and 1,500 mg/kg, respectively, while the high and low concentrations administered in the feed to both rats and mice were 44,000 and 22,000 ppm, respectively. The compound was administered for a period of 78 weeks, followed by an observation period of 26 weeks for rats and 25 weeks for mice.

There was a significant positive association between the administration of 3,3'-dimethoxybenzidine-4,4'-

diisocyanate and mortality in male and female rats, but not in male or female mice. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

For both sexes of rats, there was a significant positive association between dosage and the incidence of leukemia and malignant lymphoma. There was a significantly higher incidence of neoplasms of the skin, excluding skin of the ear, when dosed male rats were compared to controls. There was a significant positive association between the dosages administered and the incidences of endometrial stromal polyps in female rats. None of the statistical tests for any site in male and female mice indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, administration of 3,3'-dimethoxybenzidine-4,4'-diisocyanate was carcinogenic to Fischer 344 rats, causing neoplasms of the skin (excluding skin of the ear) in males, endometrial stromal polyps in females, and leukemia and malignant lymphoma in both sexes. The compound was also associated with the development of a combination of squamous-cell carcinomas and sebaceous adenocarcinomas of the Zymbal's gland and skin of the ear in rats of both sexes. There was no evidence of the carcinogenicity of the compound in $B6C3F_1$ mice.

Synonyms: 4,4'-diisocyanato-3,3'-dimethoxy-1,1'-biphenyl; dianisidine diisocyanate; isocyanic acid 3,3'-dimethoxy-4,4'-biphenylene ester; 4,4'-diisocyanato-3,3'-dimethoxy-4,4'-biphenyl diisocyanate; 3,3'-dimethoxy-4,4'-biphenylene diisocyanate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-129 Bioassay of Trimethylthiourea for Possible Carcinogenicity (CAS No. 2489-77-2)

Trimethylthiourea, useful in a wide variety of industrial applications, was selected for bioassay by the National Cancer Institute because it is a derivative of thiourea, a liver carcinogen in Osborne-Mendel rats.

A bioassay for the possible carcinogenicity of trimethylthiourea was conducted using Fischer 344 rats and $B6C3F_1$ mice. A mixture containing 80 percent trimethylthiourea and 15 percent dimethylthiourea was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of trimethylthiourea were, respectively, 500 and 250 ppm for rats and 1,000 and 500 ppm for mice. The compound was administered in the diet for 77 weeks, followed by an observation period of 29 weeks for rats and 14 weeks for mice.

There were no significant positive associations between the dosage of trimethylthiourea administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. For high dose female rats and for dosed mice of both sexes, compound-related mean body weight depression was observed, indicating that the dosages of trimethylthiourea administered to these animals may have approximated the maximum tolerated dosages. Since no mean body weight depression relative to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of trimethylthiourea to male rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

The incidences of follicular-cell carcinomas of the thyroid in female rats were dose-related, and there was a significant difference between the incidences in the high dose and control. This same relationship was established for the combination of follicular-cell carcinomas and follicular-cell adenomas in female rats.

Under the conditions of this bioassay, dietary administration of trimethylthiourea was carcinogenic in female Fischer 344 rats, inducing follicular-cell carcinomas of the thyroid. There was not sufficient evidence for the carcinogenicity of the compound in male Fischer 344 rats or in $B6C3F_1$ mice of either sex.

Synonyms: trimethylthiourea, N,N,N'-trimethylthiourea; 1,1,3-trimethyl-2-thiourea

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-130 Bioassay of Aniline Hydrochloride for Possible Carcinogenicity (CAS No. 142-04-1)

Aniline hydrochloride, a dye intermediate and a commercially important salt of aniline, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer among workers in the dye manufacturing industry and the historical association of aromatic amines with this increased cancer risk.

A bioassay of aniline hydrochloride for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. Aniline hydrochloride was administered in the feed, at either of two concentrations, to groups of 50 male and female animals of each species, with the excep-

tion of 49 female mice in the high dose group. The high and low dietary concentrations of aniline hydrochloride were, respectively, 0.6 and 0.3 percent for rats and 1.2 and 0.6 percent for mice. After a 103-week period of compound administration, observation of the rats and mice continued for up to an additional 5 weeks.

For rats and mice, respectively, 25 and 50 animals of each sex were placed on test as controls and fed only the basal diet.

In male rats there were several types of mesenchymal tumors, primarily of the spleen, associated with administration of the compound. Hemangiosarcomas of the spleen and the combined incidence of fibrosarcomas and sarcomas NOS of the spleen were each statistically significant in male rats. The combined incidence of fibrosarcomas and sarcomas NOS of multiple body organs was also significant in male rats. The number of female rats having fibrosarcomas or sarcomas NOS of either the spleen alone or multiple organs of the body cavity was significantly associated with increased dietary concentration of aniline hydrochloride. This result was not supported by the Fischer exact tests, but because of the rarity of these tumors, the observed incidences (0/24 in the control group, 1/50 [2 percent] in the low dose group, 7/50 [14 percent] in the high dose group) were considered indicative of a compound-related carcinogenic effect.

In mice of both sexes no tumors occurred in statistically significant increased incidences among dosed groups when compared to controls.

Under the conditions of this bioassay, dietary administration of aniline hydrochloride was carcinogenic to male and female Fischer 344 rats, inducing hemangiosarcomas and a combination of fibrosarcomas and sarcomas NOS of the spleen and a combination of fibrosarcomas and sarcomas NOS of multiple body organs. There was no evidence of compound-induced carcinogenicity in $B6C3F_1$ mice of either sex.

Synonyms: hydrochloride benzeneamide; aniline salt; aniline chloride

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-131 Bioassays of DDT, TDE, and p,p'-DDE for Possible Carcinogenicity (CAS No. 50-29-3, CAS No. 72-54-8, CAS No. 72-55-9)

DDT is the common name for the technical product of which p,p'-DDT is the predominant component. The compound is a synthetic, chlorinated hydrocarbon insecticide which has broad-spectrum insecticidal activity. After being used commercially and in large quantities in the United States for more than two decades, its status as an insecticide began to fade in the mid-1960s when environmentalists detected a possible link between DDT and various ecological disturbances including the decline of selected bird populations and numerous instances of fish kills.

TDE was introduced commercially in the United States in 1945 shortly after the introduction of DDT. Although lacking the broad-spectrum insecticidal activity of DDT, TDE does possess equal or greater potency against the larvae of some mosquitos and lepidoptera.

Bioassays of technical-grade DDT, TDE, and p,p'-DDE for possible carcinogenicity were conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Each compound was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each species and sex were placed on test as controls for the bioassay of each compound. The time-weighted average high and low dietary concentrations of DDT were, respectively, 642 and 321 ppm for male rats, 420 and 210 ppm for female rats, 44 and 22 ppm for male mice, and 175 and 87 ppm for female mice. The time-weighted average high and low dietary concentrations of TDE were, respectively, 3,294 and 1,647 ppm for male rats, 1,700 and 850 ppm for female rats, and 822 and 411 ppm for male and female mice. The time-weighted average high and low dietary concentrations of DDE were, respectively, 839 and 437 ppm for male rats, 462 and 242 ppm for female rats, and 261 and 148 ppm for male and female mice. After the 78-week dosing period there was an additional observation period of up to 35 weeks for rats and 15 weeks for mice.

There were significant positive associations between increased chemical concentration and accelerated mortality in female mice dosed with DDT and in both sexes of rats and in female mice dosed with DDE. This association was not demonstrated in other groups. There was, however, poor survival among control and dosed male mice used in the bioassays of DDT and DDE. In all cases adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

When those male rats receiving TDE and their controls were combined within each group so that the numerators of the tumor incidences represented those animals with either a follicular-cell carcinoma or a follicular-cell adenoma of the thyroid, the incidence in the low dose group was significantly higher than that in the controls. There was a significant positive association between the concentration of DDE administered and the incidences of hepatocellular carcinomas in male and female mice. Among dosed rats and mice no other neoplasms occurred in statistically significant incidences when compared to their respective control groups.

Under the conditions of these bioassays there was no evidence for the carcinogenicity of DDT in Osborne-Mendel rats or $B6C3F_1$ mice, of TDE in female Osborne-Mendel rats or $B6C3F_1$ mice of either sex, or of p,p'-DDE in Osborne-Mendel rats, although p,p'-DDE was hepato-

toxic in Osborne-Mendel rats. The findings suggest a possible carcinogenic effect of TDE in male Osborne-Mendel rats, based on the induction of combined follicular-cell carcinomas and follicular-cell adenomas of the thyroid. Because of the variation of these tumors in control male rats in this study, the evidence does not permit a more conclusive interpretation of these lesions. p,p'-DDE was carcinogenic in B6C3F$_1$ mice, causing hepatocellular carcinomas in both sexes.

Synonyms for DDT: 1,1'-(2,2,2-trichloroethylidene) bis(4-chloro)-benzene; 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane; p,p'-dichlorodiphenyltrichloroethane

Synonyms for TDE: 1,1'-(2,2-dichloroethylidene) bis(4-chloro)-benzene; 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethane; p,p'-dichlorodiphenyldichloroethane

Synonyms for p,p'-DDE: 1,1'-(2,2-dichloroethenylidine) bis(4-chloro)benzene; 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene; p,p'-dichlorodiphenyldichloroethylene

Report Date: 1978

Levels of Evidence of Carcinogenicity:

For p,p'-DDE (72-55-9):
Male Rats: Negative
Female Rats: Negative
Male Mice: Positive
Female Mice: Positive

For DDT (50-29-3):
Male Rats: Negative
Female Rats: Negative
Male Mice: Negative
Female Mice: Negative

For TDE (72-54-8):
Male Rats: Equivocal
Female Rats: Negative
Male Mice: Negative
Female Mice: Negative

TR-132 Bioassay of 2,5-Dithiobiurea for Possible Carcinogenicity (CAS No. 142-46-1)

2,5-Dithiobiurea, a component of photographic chemicals, was selected for bioassay by the National Cancer Institute because it is a dimer of thiourea, a liver, thyroid and Zymbal's gland tumorigen in rats.

A bioassay of 2,5-dithiobiurea for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F$_1$ mice. 2,5-Dithiobiurea was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species, with the exception of high dose male rats, of which there were only 49. The dietary concentrations used in the chronic bioassay were 0.6 percent for the low dose rats and 1.2 percent for the high dose rats. The dietary concentrations used for low and high dose mice were 1.0 and 2.0 percent, respectively. After a 78-week dosing period, observation of the mice continued for an additional 16 weeks. For each species, 50 animals of each sex were placed on test as controls.

In both species, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Compound-related mean body weight depression was observed in mice but not in rats. No consistent pattern of clinical signs was observed in either species.

No tumors occurred at a significantly higher incidence in dosed rats than in their controls. pmAmong female mice, the Cochran-Armitage test indicated a significant positive association between the incidence of hepatocellular carcinoma and dietary concentrations of 2,5-dithiobiurea. According to results of the Fisher exact test, the incidence of hepatocellular carcinoma was significantly higher in the high dose female mouse group when compared to the corresponding control group but not when compared to the laboratory historical control data. No neoplasms occurred at a significantly higher incidence in dosed male mice than in their controls.

Under the conditions of this bioassay, the evidence suggested, but was insufficient to establish the carcinogenicity of 2,5-dithiobiurea for female B6C3F$_1$ mice. The compound was not carcinogenic to male B6C3F$_1$ mice or to male or female Fischer 344 rats.

Synonym: 1,2-hydrazinedicarbothioamide

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats: Negative
Female Rats: Negative
Male Mice: Negative
Female Mice: Equivocal

TR-133 Bioassay of 3-Nitro-p-Acetophenetide for Possible Carcinogenicity (CAS No. 1777-84-0)

3-Nitro-p-acetophenetide, a derivative of the analgesic phenacetin, was selected for bioassay by the National Cancer Institute because of the suspected renal pelvic carcinogenicity of the parent compound.

A bioassay for possible carcinogenicity of 3-nitro-p-acetophenetide was conducted using Fischer 344 rats and B6C3F$_1$ mice. 3-Nitro-p-acetophenetide was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species, with the exception of low dose male mice, of which there were 49. Fifty animals of each sex and species were placed on test as controls. The high and low time-weighted average dietary concentrations of 3-nitro-p-acetophenetide were, respectively, 0.36 and 0.18 percent for rats and 1.46 and 0.73 percent for mice. The compound was administered in the diet for 78 weeks,

followed by an observation period of up to 30 weeks for rats and 20 weeks for mice.

There were no significant positive associations between the concentrations of 3-nitro-p-acetophenetide administered and mortality in rats or mice of either sex. In addition, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

There was a statistically significant increased incidence of a combination of hepatocellular carcinomas and adenomas when high dose male mice were compared to controls. No other neoplasm in any other dosed group occurred in significant positive increased incidences when compared to controls.

Under the conditions of this bioassay, dietary administration of 3-nitro-p-acetophenetide was not carcinogenic in Fischer 344 rats of either sex or in female mice. The compound, however, was considered carcinogenic in male B6C3F$_1$ mice based on a significant increase in the combined incidence of hepatocellular carcinomas and hepatocellular adenomas in these animals.

Synonyms: N-(4-ethoxyphenyl)-3'-nitroacetamide; 3'-Nitro-p-acetophenetide; 3'-Nitro-p-acetophenetidin; 4-Acetamino-2-nitrophenetole

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Negative

TR-134 Bioassay of C.I. Vat Yellow 4 for Possible Carcinogenicity (CAS No. 128-66-5)

C.I. vat yellow 4, a commercial formulation containing dibenzo(b,def)chrysene-7,14-dione, is used by the armed services as a smokescreen and as a signaling agent. Smoke dyes were effectively used to mask the movement of troops in World War II and in Korea, where in some instances men were exposed to chemical smokescreens for several months.

A bioassay of C.I. vat yellow 4 for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F$_1$ mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered C.I. vat yellow 4 in the diet at one of two doses, either 3,500 or 7,000 ppm for the rats, either 25,000 or 50,000 ppm for male mice, and either 12,500 or 25,000 ppm for the female mice. The rats were administered the test chemical for 104 weeks; the mice, for 106 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of the period of administration of the test chemical.

Mean body weights of the dosed rats were lower than those of corresponding controls throughout the bioassay, but the differences in weights were slight for the males. Mean body weights of the dosed mice were not affected by the test chemical. Survival of the rats and mice were not affected adversely by the chemical, and sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors.

In the male and female rats and the female mice, no tumors occurred at incidences that were significantly higher in dosed groups than in control groups.

In the male mice, lymphomas occurred at incidences that were dose related ($P = 0.002$) and, in a direct comparison, the incidence of the tumor in the high-dose group was significantly higher ($P = 0.019$) than that in the control group (controls 3/20, or 15%; low-dose 7/47, or 15%; high-dose 22/50, or 44%). The incidence of lymphomas and leukemias in historical-control male B6C3F$_1$ mice, at this laboratory was 38/323 (12%).

It is concluded that under the conditions of this bioassay, the formulated product containing C.I. vat yellow 4 was not carcinogenic for male or female Fischer 344 rats or for female B6C3F$_1$ mice, but was carcinogenic for male B6C3F$_1$ mice, causing an increased incidence of lymphomas.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Negative

TR-135 Bioassay of Malaoxon for Possible Carcinogenicity (CAS No. 1634-78-2)

A bioassay of malaoxon, the oxygen analogue of malathion (an organophosphate insecticide), for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were fed diets containing 500 or 1,000-ppm malaoxon for 103 weeks and were then observed for up to an additional 2 weeks. Matched controls consisted of groups of 50 untreated rats and 50 untreated mice of each sex. All surviving animals were killed at 103 to 105 weeks.

The only effects that could be related to administration of malaoxon at the doses used were increased mortality among male mice, decreased mean body weights of female mice, gastric ulcers in male and female rats, and possibly C-cell adenomas or carcinomas of the thyroid among treated female rats. The incidence of C-cell adenomas or carcinomas among historical controls, however, precluded relating the incidence of these tumors to administration of the chemical.

It was concluded that under the conditions of this bioassay malaoxon was not carcinogenic for F344 rats or $B6C3F_1$ mice.

Synonym: o,o-dimethyl S-1,2-bis(ethoxycarbonyl)ethyl phosphorothioate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-136 Bioassay of Aldicarb for Possible Carcinogenicity (CAS No. 116-06-3)

The carbamate pesticide aldicarb is used for the control of insects, nematodes, and mites. It is now registered for use on cotton, sugar beets, sugar cane, potatoes, peanuts, and a variety of field- and nursery-grown ornamental plants.

A bioassay of aldicarb for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered aldicarb at one of two doses, either 2 or 6 ppm, for 103 weeks and then observed for an additional 0 to 2 weeks. Matched controls consisted of 25 untreated rats and 25 untreated mice of each sex. All surviving animals were killed at weeks 103 to 105.

Mean body weights of the dosed male and female rats were essentially the same as those of the corresponding controls. Mean body weights of the dosed male and female mice also were essentially the same as those of corresponding controls. Hyperactivity was noted in the dosed groups of mice. Survival was not affected significantly in dosed groups of either the rats or the mice and was 72% or greater in all dosed or control groups at week 90. Sufficient numbers of animals were at risk for the development of late-appearing tumors.

No tumors occurred in either the rats or mice at incidences that could clearly be related to administration of the test chemical. In both rats and mice, however, there was no indication either through weight depression or early mortality that maximum tolerated dose levels were used. Therefore, the studies may not have been conducted using maximum sensitivity for the assessment of the possible carcinogenicity of aldicarb.

It is concluded that under the conditions of this bioassay, technical-grade aldicarb was not carcinogenic for F344 rats or $B6C3F_1$ mice of either sex.

Synonym: 2-methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-137 Bioassay of Diazinon for Possible Carcinogenicity (CAS No. 333-41-5)

Diazinon is used as an organophosphate insecticide. It was marketed first in 1954 as an insecticide and acaricide and has been used since that time as a dust or spray in agriculture, on rangeland and wasteland, in industrial establishments, and in the home. Diazinon has also been applied as a livestock spray or dip and has been administered in the feed to farm and domestic animals for the control of ectoparasites.

A bioassay of diazinon for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered diazinon at one of two doses, either 400 or 800 ppm for the rats and either 100 or 200 ppm for the mice, for 103 weeks and were then observed for an additional 1 or 2 weeks. Matched controls consisted of groups of 25 untreated rats and 25 untreated mice of each sex. All surviving animals were killed at the end of 104 or 105 weeks.

There was no appreciable effect of administration of diazinon on mean body weights of rats or mice of either sex. Mortality was not increased in any of the dosed groups of rats or mice, when related to that in the corresponding controls, and survival was 84% or greater in all dosed and control groups of animals at week 78. Some hyperactivity was noted in the dosed groups of both species; however, both the rats and mice may have been able to tolerate higher doses. Sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors.

No tumors occurred in any of the dosed groups of rats or mice of either sex at incidences that could clearly be related to the administration of diazinon.

It is concluded that under the conditions of this bioassay, diazinon was not carcinogenic for F344 rats or $B6C3F_1$ mice of either sex.

Synonym: o,o-diethyl o-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-138 Bioassay of Sulfisoxazole for Possible Carcinogenicity (CAS No. 127-69-5)

Sulfisoxazole is an antimicrobial drug that is a derivative of sulfanilamide. Although the use of sulfonamide drugs has declined in the past few years due to the emergence of drug-resistant strains of bacteria and the development of newer antimicrobial drugs with fewer side effects, these compounds are still widely prescribed on a chronic basis for the treatment of recurrent urinary tract infections and certain other infectious diseases.

A bioassay of sulfisoxazole for possible carcinogenicity was conducted by administering the chemical by gavage to Fischer 344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered sulfisoxazole suspended in aqueous 0.5% carboxymethyl cellulose 7 days per week at one of two doses, either 100 or 400 mg/kg body weight for the rats and either 500 or 2,000 mg/kg for the mice. Vehicle controls consisted of groups of 50 rats of each sex and 50 mice of each sex that were administered only the aqueous 0.5% carboxymethyl cellulose. Untreated controls consisted of groups of 50 rats of each sex and 50 mice of each sex. The dosed groups of the rats and mice were administered the chemical by gavage for 103 weeks, then observed for 1 to 3 additional weeks; the vehicle-control groups were similarly administered 0.5% carboxymethyl cellulose alone. All surviving rats and mice were killed at weeks 104 to 106.

Mean body weights of high-dose male rats and female mice were slightly lower than those of corresponding vehicle controls during the last 40 to 50 weeks of the bioassay; mean body weights of dosed female rats and male mice were unaffected. Survival rates were unaffected by the test chemical, and adequate numbers of animals were at risk for the development of late-appearing tumors.

No tumors occurred in the dosed groups of rats or mice of either sex at incidences that were significantly higher than those of the vehicle-control groups.

It is concluded that under the conditions of this bioassay, sulfisoxazole was not carcinogenic for either Fischer 344 rats or $B6C3F_1$ mice.

Synonym: N^1-(3,4-dimethyl-5-isoxazolyl)sulfanilamide

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-139 Bioassay of Triphenyltin Hydroxide for Possible Carcinogenicity (CAS No. 76-87-9)

Triphenyltin hydroxide, an organometallic compound used as a fungicide and antifeeding compound for insect control, was selected for bioassay by the National Cancer Institute because of its use on edible crops and a lack of adequate chronic toxicity data.

A bioassay of triphenyltin hydroxide for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. Triphenyltin hydroxide was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low concentrations of triphenyltin hydroxide were, respectively, 75 and 37.5 ppm for rats and mice. After a 78-week period of compound administration, there was an additional observation period of 26 weeks for both species. Twenty animals of each sex and species were placed on test as controls.

For male mice, there was a significant positive association between dosage and mortality. In both species, however, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. Except for a slight depression of mean body weight gain in male rats and female mice, compound-related mean body weight depression was not observed in either species. In female rats no significant accelerated mortality, retardation of growth, or other signs of toxicity were associated with the dietary administration of triphenyltin hydroxide. Therefore, it is possible that the compound was not administered at the maximum tolerated concentrations.

No tumors occurred at a significantly higher incidence in dosed rats or mice than in controls.

Under the conditions of this bioassay, there was no evidence for the carcinogenicity of triphenyltin hydroxide to Fischer 344 rats or $B6C3F_1$ mice.

Synonyms: hydroxytriphenylstannane; hydroxytriphenyltin; fentin hydroxide; TPTH; TPTOH; ENT 2009

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-140 Bioassay of Pivalolactone for Possible Carcinogenicity (CAS No. 1955-45-9)

Pivalolactone, an intermediate in polymer preparation, was selected for bioassay by the National Cancer Institute because of the structural similarity of this compound to β-propiolactone, a well documented direct acting carcinogen.

The bioassay of pivalolactone for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. Pivalolactone in water was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The high and low

dosages of pivalolactone utilized were, respectively, 300 and 150 mg/kg/day for rats and 150 and 75 mg/kg/day for mice. After a 103-week period of compound administration for rats and a 102-week period of compound administration for mice, rats were observed for 2 additional weeks and mice for 1 additional week. Twenty animals of each sex and species were placed on test as vehicle controls.

There was no significant positive association between dosage and mortality for either rats or mice, and in both species, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. Compound-related mean body weight depression was not observed in either sex of either species. In addition, no adverse clinical signs were observed among dosed mice. This evidence, plus the relatively fast decomposition of pivalolactone in water, suggests the possibility that the animals, and in particular the mice, may have been able to tolerate a higher dose.

Statistically significant incidences of squamous-cell papillomas and squamous-cell carcinomas of the forestomach were observed in rats but not in mice. No other rare or unusual tumors were observed in either species.

Under the conditions of this bioassay, pivalolactone was found to be carcinogenic to both male and female Fischer 344 rats, producing squamous-cell carcinomas and squamous-cell papillomas of the forestomach. This study provided no evidence for the carcinogenicity of pivalolactone in $B6C3F_1$ mice of either sex.

Synonyms: 3,3-dimethyl-2-oxethanone; 3,3-dimethyl-2-oxetanone; 3,3-dimethyl-β-propiolactone; dimethyl propiolactone; pivalic acid lactone

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-141 Bioassay of 1-Phenyl-3-methyl-5-pyrazolone for Possible Carcinogenicity (CAS No. 89-25-8)

1-Phenyl-3-methyl-5-pyrazolone, an aromatic heterocycle and widely used dye intermediate, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer observed among workers in the dye manufacturing industry.

A bioassay of 1-phenyl-3-methyl-5-pyrazolone for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. 1-Phenyl-3-methyl-5-pyrazolone was administered in the feed, at either of two concentrations, to groups of 49 or 50 male and 50 female animals of each species. The high and low concentrations of 1-phenyl-3-methyl-5-pyrazolone utilized were, respectively, 5,000 and 2,500 ppm for rats and 15,000 and 7,500 ppm for mice. Twenty animals of each species and sex were placed on test as controls. After a 103-week period of chemical administration, there was an additional observation period of 2 weeks for rats. A 102-week period of chemical administration was followed by an additional 2-week observation period for mice.

In both species adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. Compound-related mean body weight depression was observed in mice, but not in rats. In addition, no significant accelerated mortality or other signs of toxicity were associated with the dietary administration of 1-phenyl-3-methyl-5-pyrazolone to rats; therefore, it is possible that the compound was not administered to rats at the maximum tolerated concentration.

There were no tumors in either sex of rats or mice for which a significant positive association could be established between chemical administration and incidence.

Under the conditions of this bioassay, there was no evidence for the carcinogenicity of 1-phenyl-3-methyl-5-pyrazolone to Fischer 344 rats or $B6C3F_1$ mice.

Synonyms: 2,4 - dihydro - 5 - methyl - 2 - phenyl - 3H - pyrazol-3-one; 3-methyl-1-phenyl-2-pyrazolin-5-one; phenyl-3-methylpyrazolone; 1-phenyl-3-methyl-5-oxo-2-pyrazoline; 1-phenyl-5-(3-methylpyrazolone); norphenazone; Developer Z; C.I. Developer 1

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-142 Bioassay of p-Cresidine for Possible Carcinogenicity (CAS No. 120-71-8)

p-Cresidine, used in the production of various azo dyes, was selected for bioassay by the National Cancer Institute in response to the high incidence of bladder cancer observed among dye manufacturing industry workers. Aromatic amines are one class of chemicals believed to contribute to the increased cancer risk in this industry.

A bioassay of p-cresidine for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. p-Cresidine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The dietary concentrations used in the chronic bioassay for low and high dose rats were 0.5 and 1.0 percent, respectively. The time-weighted average concentrations fed to low dose male, low dose female, high dose male and high dose female mice were 0.22, 0.22. 0.46, and 0.44 percent, respectively.

All dosed animals, except for high dose male mice, were administered p-cresidine in the diet for 104 weeks

and observed for an additional period of up to 2 weeks. All high dose male mice were dead by the end of week 92. For each species, 50 animals of each sex were placed on test as controls and fed only the basal laboratory diet.

Mortality rates were dose-related for both sexes of both species. That incidences of certain tumors were higher in low dose than in high dose groups was probably due to accelerated mortality in the high dose group.

In dosed rats of both sexes, statistically significant incidences of bladder carcinomas (combined incidences of papillary carcinomas, squamous-cell carcinomas, transitional-cell papillomas, transitional-cell carcinomas, and undifferentiated carcinomas) and olfactory neuroblastomas were observed. The combined incidence of neoplastic nodules of the liver, hepatocelular carcinomas, or mixed hepato/cholangio carcinomas was also significant in low dose male rats.

In both male and female dosed mice, the incidence of bladder carcinomas (combined incidence of carcinomas NOS, squamous-cell carcinomas, and transitional carcinomas) was significant. The incidence of hepatocellular carcinomas was also significant in dosed female mice.

Under the conditions of this bioassay, p-cresidine was *carcinogenic to Fischer 344 rats, causing increased incidences* of carcinomas and of papillomas of the urinary bladder in both sexes, increased incidences of olfactory neuroblastomas in both sexes, and of liver tumors in males. p-Cresidine was also carcinogenic in $B6C3F_1$ mice, causing carcinomas of the urinary bladders in both sexes and hepatocellular carcinomas in females.

Synonyms: 2-methoxy-5-methylbenzeneamine; 2-methoxy- 5-methylaniline; 5-methyl-o-anisidine; m-amino-p-cresol methyl ether; MASO; cresidine

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-143 Bioassay of 1,5-Naphthalenediamine for Possible Carcinogenicity (CAS No. 2243-62-1)

1,5-Naphthalenediamine, a bicyclic aromatic amine used in the dye industry, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer reported among dye manufacturing workers. Aromatic amines are one of a class of chemicals believed to contribute to the increased cancer risk in this industry.

A bioassay of 1,5-naphthalenediamine for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. 1,5-Naphthalenediamine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low dietary concentrations utilized in the chronic bioassay were, respectively, 0.1 and 0.05 percent for rats and 0.2 and 0.1 percent for mice. The compound was administered in the diet for 103 weeks, followed by up to 4 weeks of observation. Fifty mice of each sex and 25 rats of each sex were placed on test as controls. These animals were observed for up to 110 weeks.

There were no significant positive associations between the administered concentrations of 1,5-naphthalenediamine and mortality in either sex of rats or mice. In all groups adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors.

Among dosed female rats, a statistically significant increase in endometrial stromal polyps was observed. Several of these tumors underwent malignant transformation to endometrial stromal sarcomas. The incidence of female rats having either adenoma or carcinoma of the clitoral gland was statistically significant. No neoplasms were observed at significantly increased incidences in dosed male rats. Based on lack of clinical signs or weight loss, the male rats may have been able to withstand a higher dose.

In mice, dose-related increases in thyroid neoplasms were observed in both sexes. The incidence of thyroid C-cell carcinomas was significant for high dose female mice. The combined incidences of papillary adenomas, follicular-cell adenomas and papillary cystadenomas of the thyroid were significant for mice of both sexes. The incidence of hepatocellular carcinomas and the incidence of alveolar/bronchiolar adenomas were each significant for dosed female mice.

Under the conditions of this bioassay, 1,5-naphthalenediamine was carcinogenic in female Fischer 344 rats, causing clitoral and uterine neoplasms. 1,5-Naphthalenediamine was also carcinogenic for $B6C3F_1$ mice, producing thyroid neoplasms in males and neoplasms of the thyroid, liver, and lung in females. Insufficient evidence was provided for the carcinogenicity of the compound in male Fischer 344 rats.

Synonym: 1,5-diaminonaphthalene

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-144 Bioassay of 2-Aminoanthraquinone for Possible Carcinogenicity (CAS No. 117-79-3)

2-Aminoanthraquinone, an intermediate in the synthesis of anthraquinone dyes, was selected for bioassay by the National Cancer Institute in an attempt to determine which chemicals may be responsible for the

increased incidence of bladder cancer observed among workers in the dye manufacturing industry. Aromatic amines are one of several classes of chemicals thought to contribute to the increased cancer risk in this industry.

A bioassay of 2-aminoanthraquinone for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. 2-Aminoanthraquinone was administered in the feed, at either of two concentrations (except for female rats), to groups of 50 male and 50 female animals of each species. The time-weighted average dietary concentrations used in the chronic bioassay were 0.69 and 0.35 percent for high and low dose male rats, respectively, 0.2 percent for the treated female rats, and 1.0 and 0.5 percent, respectively, for high and low dose mice of both sexes. After a 78-week period of chemical administration (80 weeks for high dose mice), observation of the rats continued for up to an additional 32, weeks and observation of the mice continued for up to an additional 16 weeks.

In both species adequate numbers of animals in all groups, except the treated female rats, survived sufficiently long to be at risk from late-developing tumors. The survival among treated female rats was poor and, as a result, no conclusions could be made regarding the carcinogenicity of the compound in these animals.

When male rats having either hepatocellular carcinomas or neoplastic nodules of the liver were combined and the resulting tumor incidences were analyzed statistically, there was a significant positive association between dosage and the incidences of these combined neoplasms. Hepatocellular carcinomas were observed at significantly higher incidences when dosed mice were compared to controls. There was a significantly higher incidence of malignant hematopoietic lymphomas in high dose female mice when compared to controls.

Under the conditions of this bioassay, dietary administration of 2-aminoanthraquinone was carcinogenic in male Fischer 344 rats, causing a combination of hepatocellular carcinomas and neoplastic nodules of the liver. The compound was also carcinogenic in $B6C3F_1$ mice, causing hepatocellular carcinomas in both sexes and malignant hematopoietic lymphomas in females.

Synonyms: 2-amino-9,10-anthracenedione; AAQ

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Inadequate Study
Male Mice:	Positive
Female Mice:	Positive

TR-145 Bioassay of 3-Chloro-p-Toluidine for Possible Carcinogenicity (CAS No. 95-74-9)

3-Chloro-p-toluidine, a dye intermediate and avicide, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer observed among workers in the dye manufacturing industry. Aromatic amines, of which 3-chloro-p-toluidine is one example, are among several classes of chemicals believed to contribute to this increased cancer risk.

A bioassay for the possible carcinogenicity of 3-chloro-p-toluidine was conducted using Fischer 344 rats and $B6C3F_1$ mice. 3-Chloro-p-toluidine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The time-weighted average dietary concentrations of 3-chloro-p-toluidine administered to rats of both sexes were 3,269 and 1,635 ppm for the high and low dose groups, respectively. The high and low dietary concentrations of 3-chloro-p-toluidine administered to mice were, respectively, 1,200 and 600 ppm for males and 600 and 300 ppm for females. The compound was administered in the diet for 78 weeks, followed by an observation period of 24 weeks for high dose male rats, 25 weeks for all other dosed rats, and 12 weeks for mice.

There were no significant positive associations between the concentrations of 3-chloro-p-toluidine administered and mortality in either species. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression, relative to controls, was observed in high dose rats and mice of both sexes, indicating that the concentrations administered to these animals may have approximated the maximum tolerated dosages. The unusual incidences of nonneoplastic spleen and liver lesions in high dose rats supports this assumption.

Under the conditions of this bioassay there was no convincing evidence for the carcinogenicity of 3-chloro-p-toluidine in Fischer 344 rats or $B6C3F_1$ mice.

Synonyms: 3-chloro-4-methylbenzeneamine; 1-amino-3-chloro-4-methylbenzene; CPT

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-146 Bioassay of Nithiazide for Possible Carcinogenicity (CAS No. 139-94-6)

Nithiazide, an antiprotozal compound used in veterinary medicine, was selected for bioassay by the National Cancer Institute because of its use and possible persistence in the tissues and eggs of animals raised for human consumption.

The bioassay of nithiazide for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. Nithiazide was administered in the diet, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low concentrations of nithiazide utilized were, respectively, 1,250 and 625 ppm for rats and 5,000 and 2,500 ppm for mice. Dosed rats received feed containing nithiazide for 38 weeks, and as a result of a shortage of nithiazide, the animals were not fed the dosed feed for the next 9 weeks. The dosed feed diet was then resumed and continued for 56 weeks, after which time a 1-week observation period followed. Dosed mice received feed containing nithiazide for 61 weeks and, due to a shortage of nithiazide, the animals were not fed dosed feed for the next 9 weeks. The dosed feed diet was then resumed and continued for 33 weeks, followed by a 1-week observation period. Twenty animals of each sex and species were placed on test as controls.

In both species, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. There was no significant positive association between dosage and mortality for either rats or mice. Compound-related mean body weight depression occurred in both sexes of each species.

Statistically significant incidences of hepatocellular adenomas and carcinomas were found in high dose male mice but not in female mice. Although the increased incidences of these tumors in dosed female mice were not statistically significant, the evidence presented was strongly suggestive of carcinogenicity to the liver in female $B6C3F_1$ mice. Statistically significant increased incidences of a combination of mammary and skin fibroadenomas and cystadenomas NOS were found in the high dose female rats. No unusual tumors were observed in either species.

Under the conditions of this bioassay, nithiazide was carcinogenic in male and probably female $B6C3F_1$ mice, causing a combination of hepatocellular carcinomas and hepatocellular adenomas. Nithiazide was also carcinogenic in female Fischer 344 rats, causing an increase in the incidence of mammary neoplasms. The compound was not carcinogenic in male Fischer 344 rats.

Synonyms: N-ethyl-N'-(5-nitro-2-thiazolyl) urea; 1-ethyl-3-(5-nitro-2-thiazolyl) urea

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Equivocal

TR-147 Bioassay of Mexacarbate for Possible Carcinogenicity (CAS No. 315-18-4)

Mexacarbate is one of a group of agricultural pesticides that scientists at the National Cancer Institute noted, in the late 1960's, had not been adequately tested for carcinogenicity. Mexacarbate has been used as an insecticide and as a molluscicide for the control of pests on lawns, turf, and flowers.

A bioassay of technical-grade mexacarbate for possible carcinogenicity was conducted using Osborne-Mendel rats and $B6C3F_1$ mice. Mexacarbate was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The time-weighted average high and low dietary concentrations of mexacarbate were 418 and 209 ppm for male rats, 678 and 339 ppm for female rats, 654 and 327 ppm for male mice and 135 and 68 ppm for female mice. After a 78-week period of chemical administration, observation of rats continued for an additional 33 to 34 weeks and observation of mice continued for 14 to 15 additional weeks. For each species, 20 animals of each sex were placed on test as controls.

All groups except the male control mice survived sufficiently long to be at risk from late-appearing tumors. Because of poor survival of the male control mice, a pooled control group was used for statistical analysis of tumor incidence in male mice.

The possibility that female mice in this study did not receive maximum tolerated dosages of mexacarbate should be considered. Administration of mexacarbate had no significant effect on survival or body weights of female mice.

No neoplasms occurred in statistically significant increased incidences when dosed rats were compared to controls.

Among male mice surviving at least 56 weeks, significant associations with dietary concentrations were indicated by the Cochran-Armitage test for hepatocellular carcinomas, for subcutaneous fibrosarcomas and for fibromas of the skin. In none of these cases, however, were these results supported by significant Fisher exact tests.

Under the conditions of this bioassay, sufficient evidence was not obtained for the carcinogenicity of mexacarbate for Osborne-Mendel rats or $B6C3F_1$ mice.

Synonyms: 4-(dimethylamino)-3,5-dimethylphenyl methylcarbamate; 4-dimethylamino-3,5-xylyl methylcarbamate

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-148 Bioassay of 1-Phenyl-2-thiourea for Possible Carcinogenicity (CAS No. 103-85-5)

1-Phenyl-2-thiourea was selected for bioassay by the National Cancer Institute because of the structural sim-

ilarity of this compound to ethylene thiourea, a tumorigen in hybrid mice (C57BL/6 x C3H/Anf and C57BL/6 x AKR), and the widespread oral exposure to this compound when used in classroom demonstrations of genetic polymorphism in taste.

A bioassay of 1-phenyl-2-thiourea for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F$_1$ mice. 1-Phenyl-2-thiourea was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low concentrations of 1-phenyl-2-thiourea utilized in the chronic bioassay were, respectively, 120 and 60 ppm for rats and 300 and 150 ppm for mice. Twenty animals of each species and sex were placed on test as controls. A 78-week period of chemical administration was followed by an additional observation period of 26 weeks for rats and 13 weeks for mice.

Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Distinct dose-related depression of mean body weight gain was observed in male and female mice when compared with their controls, but growth retardation was not observed in any dosed rat group. In addition, since no significant accelerated mortality or other toxic effects were associated with the dietary administration of 1-phenyl-2-thiourea to rats, it is possible that the compound was not administered to these animals at the maximum tolerated concentrations.

There were no tumors in either sex of rats or mice for which a significant positive association could be established between chemical administration and tumor incidence.

Under the conditions of this bioassay, 1-phenyl-2-thiourea was not carcinogenic to Fischer 344 rats or B6C3F$_1$ mice.

Synonyms: Phenylthiourea; Phenylthiocarbamide; 1-Phenylthiourea; N-Phenylthiourea; PTU

Report Date: 1978

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-149 Bioassay of N,N'-Diethylthiourea for Possible Carcinogenicity (CAS No. 105-55-5)

N,N'-Diethylthiourea, a corrosion inhibitor and accelerator in elastomer manufacture, was selected for bioassay by the National Cancer Institute because of the structural similarity of this compound to ethylene thiourea, a tumorigen in hybrid mice (C57BL/6 x C3H/Anf and C57BL/6 x AKR).

A bioassay for the possible carcinogenicity of N,N'-diethylthiourea was conducted using Fischer 344 rats and B6C3F$_1$ mice. N,N'-Diethylthiourea was administered in the feed, at either of two concentrations, to groups of 50 males and 50 females of each species. Twenty animals of each sex and species, except for 19 male mice, were placed on test as controls. The high and low dietary concentrations of N,N'-diethylthiourea were, respectively, 250 and 125 ppm for rats and 500 and 250 ppm for mice. The compound was administered in the diet for 103 weeks, followed by an observation period of 1 week for all dosed groups.

There were no significant positive associations between the dosages of N,N'-diethylthiourea administered and mortality in rats or mice of either sex. Adequate numbers of animals in all dose groups survived sufficiently long to be at risk from late-developing tumors. Compound-related mean body weight depression was apparent among dosed male and female mice when compared to their respective controls, indicating that the concentrations of N,N'-diethylthiourea administered to mice may have approximated the maximum tolerated dosages.

There were statistically significant elevated incidences of follicular-cell carcinomas of the thyroid in high dose male rats. In addition, there were statistically significant elevated incidences of a combination of thyroid follicular-cell carcinomas and follicular-cell adenomas in high dose male and female rats.

Under the conditions of this bioassay, N,N'-diethylthiourea was carcinogenic to Fischer 344 rats, causing follicular-cell carcinomas of the thyroid in males and follicular-cell neoplasms of the thyroid in females. There was no evidence for the carcinogenicity of the compound in B6C3F$_1$ mice.

Synonyms: 1,3-diethyl-2-thiourea; 1,3-diethylthiourea

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-150 Bioassay of Butylated Hydroxytoluene (BHT) for Possible Carcinogenicity (CAS No. 128-37-0)

The phenolic antioxidant butylated hydroxytoluene (BHT) was patented in 1947 and received approval for use as a food additive and preservative by the Food and Drug Administration (FDA) in 1954. Since 1959, BHT has been generally recognized as safe (GRAS) for use in foods and is one of the most commonly used antioxidants in foods containing fats.

A bioassay of BHT for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered BHT at one of two doses, either 3,000 or 6,000 ppm; the rats for 105 weeks and the mice for 107 or 108 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of administration of the test chemical.

Mean body weights of the dosed rats and mice were lower than those of the corresponding controls and were dose related throughout most of the bioassay. Survival was not affected significantly in the dosed groups of rats or mice, and the survival was 60% or greater in all dosed or control groups of rats and mice of each sex at the end of the bioassay. Sufficient number of animals were at risk for the development of late-appearing tumors.

Alveolar/bronchiolar carcinomas or adenomas occurred in the female mice at a significant incidence in the low-dose group ($P = 0.009$) but not in the high dose group, and the incidences were not significantly dose related (control 1/20, low-dose 16/46, high-dose 7/50). Thus, these lung tumors in the female cannot clearly be related to the administration of the BHT. No tumors occurred in either male or female rats at incidences that were significantly higher in dosed groups than in corresponding control groups. Nonneoplastic lesions that may have been related to the administration of the test chemical included focal alveolar histiocytosis at increased incidences in the dosed female rats and various lesions of the liver at increased incidences in the dosed male mice.

It is concluded that under the conditions of this bioassay, BHT was not carcinogenic for F344 rats or $B6C3F_1$ mice.

Synonyms: 2,6-di-tert-butyl-p-cresol, BHT

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-151 Bioassay of Lead Dimethyldithiocarbamate for Possible Carcinogenicity (CAS No. 19010-66-3)

The lead salt of bis(dimethyldithiocarbamic) acid is used commercially as a rubber accelerator in applications involving natural rubber, and styrene-butadiene, isobutylene-isoprene, isoprene, and butadiene rubber. Dithiocarbamate accelerators are known as ultra accelerators due to their speed of reaction. They are used primarily in latexes and rubber cements.

A bioassay of technical-grade lead dimethyldithiocarbamate for possible carcinogenicity was conducted by administering the test chemical in feed to F344 (Fischer) rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered lead dimethyldithiocarbamate at one of two doses, either 25 or 50 ppm, for 104 or 105 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of the period of administration of the test chemical.

Mean body weights of the dosed male rats and female mice were slightly lower than those of the corresponding controls; mean body weights of the dosed female rats and male mice were essentially the same as those of the corresponding controls. Survival rats in both species were unaffected by administration of the test chemical. The lack of toxicity in both species suggests that a maximum tolerated dose level may not have been used. Therefore, the studies may not have been conducted using maximum sensitivity for the assessment of the possible carcinogenicity of lead dimethyldithiocarbamate.

No tumors occurred in the rats or mice of either sex at incidences that were significantly higher in the dosed groups than in the control groups.

It is concluded that under the conditions of this bioassay, lead dimethyldithiocarbamate was not carcinogenic for F344 rats or $B6C3F_1$ mice of either sex.

Synonym: ledate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-152 Bioassay of Ethyl Tellurac for Possible Carcinogenicity (CAS No. 20941-65-5)

Ethyl tellurac is used in rubber processing where it functions to accelerate the rate of vulcanization or formation of sulfur bridges between rubber polymers that produces modulus or rigidity in the finished product.

A bioassay of technical-grade ethyl tellurac for possible carcinogenicity was conducted by administering the preparation in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered ethyl tellurac at one of two doses, either 300 or 600 ppm for the males and either 150 or 300 ppm for the females, for 105 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at 105 weeks.

Groups of 50 mice of each sex were administered ethyl tellurac at one of two doses, initially either 2,500 or 5,000 ppm. Due to signs of toxicity in the dosed animals, these doses were reduced to 500 and 2,000 ppm, respectively, starting at week 41 for the males and at week 38 for the females. The reduced doses were maintained for 66 weeks for the males; for the females, the reduced doses

were raised after 3 weeks to 2,000 and 5,000 ppm, respectively, and maintained at these levels for 66 weeks. The time-weighted average doses for the males were either 1,255 or 3,132 ppm; for the females, either 2,132 or 4,915 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at 106 weeks.

Mean body weights of the dosed groups of rats or mice were lower than those of corresponding controls throughout most or all of the bioassay. No other clinical signs in the rats or mice were clearly related to administration of the test chemical. Survival of the rats and the mice was not affected by the chemical, and sufficient numbers of all groups were at risk for the development of late-appearing tumors.

In the male rats, mesotheliomas occurred at incidences that were dose related ($P = 0.012$); in direct comparisons, the incidences of the tumors in the individual dosed groups were not significantly higher than that in the control group (controls 0/20, low-dose 2/49, high-dose 8/50). However, the historical-control data at this laboratory indicate an incidence of 12/416 (2.9%) in male F344 rats compared with 8/50 (16%) in the male high-dose group in this study.

In the female rats, no tumors occurred at incidences that were related to administration of the test chemical.

In both male and female mice, adenomas of the lacrimal (harderian) gland of the eye occurred in the dosed groups, but not in the corresponding controls (males: controls 0/17; low-dose 16/46, high-dose 10/49; females: controls 0/20, low-dose 6/50, high-dose 5/49). The incidences in the dosed groups were not high enough to show statistically significant dose-related trends. However, in direct comparisons of dosed and control groups of male mice, the incidence was statistically significant in the low-dose males ($P = 0.003$). In female mice, direct comparisons of dosed and control groups indicated that the incidence of this tumor was not statistically significant.

It is concluded that under the conditions of this bioassay, ethyl tellurac was not carcinogenic for F344 rats or $B6C3F_1$ mice of either sex. The incidence of mesotheliomas in dosed male rats and the incidence of adenomas of the lacrimal (harderian) gland of the eye in dosed mice of either sex provided evidence which was suggestive but under the conditions of the bioassay insufficient to establish the carcinogenicity of ethyl tellurac in these animals.

Synonym: tellurium diethyldithiocarbamate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Equivocal

TR-153 Bioassay of *o*-Toluidine Hydrochloride for Possible Carcinogenicity (CAS No. 636-21-5)

o-Toluidine and its hydrochloride salt are dye intermediates used in the manufacture of a large number of textile dyes which include some of the azo, triarylmethane, sulfur, and indigoid compounds. In addition, there are numerous substituted *o*-toluidines that are used as dye intermediates. *o*-Toluidine also functions as a photographic dye, as a reagent in a clinical assay for glucose and hemoglobin, and as an antioxidant in the manufacture of rubber.

A bioassay of o-toluidine hydrochloride for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered *o*-toluidine hydrochloride at one of several doses, either 3,000 or 6,000 ppm for rats and either 1,000 or 3,000 ppm for the mice, for 101 to 104 weeks. Matched controls consisted of 20 untreated rats of each sex and 20 untreated mice of each sex. All surviving rats and mice were killed at the end of administration of the test chemical.

Mean body weights of dosed male and female rats and mice were lower than those of corresponding matched controls and were dose related. Mortalities of the male and female rats were dose related and were relatively high at the end of the bioassay. Mortalities of the male and female mice were not, however, significantly affected by administration of the test chemical.

In rats, the administration of the test chemical induced several types of sarcomas of the spleen and other organs in both males and females, mesotheliomas of the abdominal cavity or scrotum in males, and transitional-cell carcinomas of the urinary bladder in females. Administration of the *o*-toluidine hydrochloride also resulted in increased incidences of fibromas of the subcutaneous tissue in the males and fibroadenomas or adenomas of the mammary gland in females.

In mice, hemangiosarcomas were induced at various sites in males, and hepatocellular carcinomas or adenomas were induced in females.

Under the conditions of this bioassay, o-toluidine hydrochloride was carcinogenic in both male and female F344 rats and $B6C3F_1$ mice, producing a significant increased incidence of one or more types of neoplasms.

Synonym: 2-aminotoluene hydrochloride

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-154 Bioassay of Azobenzene for Possible Carcinogenicity (CAS No. 103-33-3)

Azobenzene occurs as a by-product during the manufacture of benzidine. Benzidine is a widely used intermediate for the azo dyes and other organic chemicals and is a carcinogen. Azobenzene itself has no known uses as a dyestuff and is only produced in small quantities for research purposes.

A bioassay of azobenzene for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered azobenzene at one of two doses, either 200 or 400 ppm, for 105 or 106 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at the end of administration of the test chemical.

Groups of 50 male mice were administered azobenzene at one of two doses, either 200 or 400 ppm, for 105 weeks. Groups of 50 female mice were administered the test chemical at one of two doses, initially 400 or 800 ppm, for 38 weeks. Because of excessively lowered body weights in the dosed groups of the females, doses for the females were then reduced to 100 and 400 ppm, respectively, and administration at the lowered doses was continued for 67 or 68 weeks. The time-weighted average doses for the female mice were either 208 or 545 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of administration of the test chemical.

Mean body weights of dosed rats and mice of each sex were lower than those of corresponding controls, and were generally dose related throughout the bioassay. Mortality was dose related in the male rats and the female mice, but was not significantly affected in either the female rats or the male mice. Survival was 70% or greater at week 90 on study in all dosed and control groups of each species and sex; thus, sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors.

In rats, a large number of sarcomas, including fibrosarcomas, hemangiosarcomas, and osteosarcomas in both males and females and malignant hemangiopericytomas in females, occurred in the spleen and other abdominal organs at incidences that were dose related in each sex ($P<0.001$) and that in direct comparisons were significantly higher ($P<0.001$) in the high-dose groups of each sex than in the corresponding control groups (males: controls, 0/20, low-dose 6/49, high-dose 31/49; females: controls 0/20, low-dose 5/50, high-dose 21/50).

In mice, no tumors occurred in either males or females at incidences that were significantly higher in the dosed groups than in the corresponding control groups.

It is concluded that under the conditions of this bioassay, azobenzene was carcinogenic (sarcomagenic) for F344 rats, inducing various types of sarcomas in the spleen and other abdominal organs of both males and females. The test chemical was not carcinogenic for $B6C3F_1$ mice of either sex.

Synonyms: diphenyldiimide; azobenzide

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-155 Bioassay of 2,4,6-Trichlorophenol for Possible Carcinogenicity (CAS No. 88-06-2)

2,4,6-Trichlorophenol is a germicidal agent that has been used to preserve wood and glue as well as to protect textiles against mildew. Production of this chemical (for sale as an end product) was discontinued in 1975 by Dow Chemical Company, the only manufacturer of 2,4,6-trichlorophenol in the United States, because of the high cost of removing toxic dioxin impurities. However, a small quantity (2,204 pounds) was imported for domestic use in 1976.

A bioassay of 2,4,6-trichlorophenol for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered 2,4,6-trichlorophenol at one of two doses, either 5,000 or 10,000 ppm, for 106 or 107 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at the end of administration of the test chemical.

Groups of 50 male mice were administered 2,4,6-trichlorophenol at one of two doses, either 5,000 or 10,000 for 105 weeks. Groups of 50 female mice were administered the test chemical at one of two doses, initially either 10,000 or 20,000 ppm, for 38 weeks. Because of excessively lowered body weights in the dosed groups of the females, the doses for the females were then reduced to 2,500 and 5,000 ppm, respectively, and administration at the lower doses was continued for 67 weeks. The time-weighted average doses for the female mice were either 5,214 or 10,428 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of administration of the test chemical.

Mean body weights of dosed rats and mice of each sex were lower than those of corresponding controls and were dose related throughout the bioassay. Survivals to the end of the experiment were 68% or greater in all groups of rats and 80% or greater in all groups of mice.

In the male rats, lymphomas or leukemias occurred at incidences that were dose related ($P = 0.006$) and in direct comparisons were significantly higher in the low-dose ($P = 0.019$) and high-dose ($P = 0.004$) groups than in the corresponding control group (controls 4/20; low-dose 25/50; high-dose 29/50). Leukocytosis and monocytosis of the peripheral blood and hyperplasia of the bone marrow also occurred in some dosed male rats not having lymphoma or leukemia.

In female rats, monocytic leukemia did not occur at incidences that were significant. However, as in the male rats, leukocytosis and monocytosis of the peripheral blood and hyperplasia of the bone marrow occurred in the dosed female rats but not in the controls (blood leukocytosis and monocytosis: controls 0/20, low-dose 6/50, high-dose 3/50; bone marrow hyperplasia: controls 0/20, low-dose 16/50, high-dose 2/50).

In both the male and female mice, hepatocellular carcinomas or adenomas occurred at incidences that were dose related ($P<0.001$), and in direct comparisons were significantly higher in the low- and high-dose male groups and the high-dose female group ($P \leq 0.001$) than in the corresponding control groups (males: controls 4/20, low-dose 32/49, high-dose 39/47; females: controls 1/20, low-dose 12/50, high-dose 24/48).

It is concluded that under the conditions of this bioassay, 2,4,6-trichlorophenol was carcinogenic in male F344 rats, inducing lymphomas or leukemias. The test chemical was also carcinogenic in both sexes of $B6C3F_1$ mice, inducing hepatocellular carcinomas or adenomas.

Synonyms: Omal®; Dowicide® 2S

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-156 Bioassay of p,p'-Ethyl-DDD for Possible Carcinogenicity (CAS No. 72-56-0)

p,p'-Ethyl-DDD, an organochlorine insecticide which is marketed under the trade name Perthane®, has a lower toxicity to both insects and mammals than its structural analogs, DDT and DDD and is of moderate persistence in the environment. First marketed in 1950 for use against houseflies and cloth moths, it has since been used on vegetables, pears, and livestock. In the late 1950's, this compound was one of several DDT analogs that were administered to patients with breast or prostatic cancer for adrenocortical suppression because of the selective toxicity of these compounds for the adrenal cortex.

A bioassay of p,p'-ethyl-DDD for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered p,p'-ethyl-DDD at one of two doses, either 3,500 or 7,000 ppm, for 105 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at the end of administration of the test chemical.

Groups of 50 male mice were administered p,p'-ethyl-DDD at one of two doses, either 2,500 or 5,000 ppm, for 105 weeks. Groups of 50 female mice were administered the test chemical at one of two doses, initially either 5,000 or 10,000 ppm. Because of excessive lowered body weights in the dosed groups of females, the doses for the females were reduced after 48 weeks to 1,000 and 3,000 ppm, respectively, and administration at the lowered doses was continued for 57 weeks. The time-weighted average doses for the female mice were 2,828 and 6,200 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of administration of the test chemical.

No tumors occurred in the male or female rats or in the male mice at incidences that could clearly be related to administration of the test chemical.

In female mice, hepatocellular carcinomas or adenomas occurred at incidences that were dose related ($P = 0.011$), but in direct comparisons the incidences in the individual dosed groups were not significantly higher than that in the corresponding control group. Although the occurrence of hepatocellular carcinomas or adenomas in the dosed female mice are not clearly related to the administration of the test chemical, the increased incidence of these tumors in the high-dose group suggests that the tumors may be related to the administration of p,p'-ethyl-DDD.

It is concluded that under the conditions of this bioassay, p,p'-ethyl-DDD was not carcinogenic for male or female F344 rats or male $B6C3F_1$ mice. However, the occurrence of hepatocellular carcinomas and adenomas in female mice was suggestive of a carcinogenic effect.

Synonym: 1,1-dichloro-2,2-bis(p-ethylphenyl)ethane

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Equivocal

TR-157 Bioassay of Methyl Parathion for Possible Carcinogenicity (CAS No. 298-00-0)

Methyl parathion is used in the agricultural industry as a contact and stomach poison with broad-spectrum insecticidal activity and some efficacy against mites. It is sold as a wettable power or emulsifiable concentrate for foliage application. Several formulations contain combinations of methyl parathion and ethyl parathion as well as other registered pesticides. There are 62 crops on which methyl parathion is registered for use, but over 90% of the total volume used in 1974 was on cotton. It is used to some extent in California for mosquito control.

A bioassay of methyl parathion for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered methyl parathion at one of two doses, initially either 62.5 or 125 ppm. These doses were maintained for 102 weeks

for the females; however, due to decreased mean body weight gain in the dosed males, the low and high doses for the males were reduced after 37 weeks to 20 and 50 ppm, respectively, and administration at the lowered doses was continued for 65 weeks. The time-weighted average doses for the male mice were 35 and 77 ppm, respectively, for the low- and high-dose groups. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of administration of the test chemical.

Mean body weights of the dosed male and female rats and mice were lower than those of the corresponding controls throughout the bioassay and were dose related. Survival was unaffected in both species except for an increase in mortality in the high-dose female rats, in which 46% of the animals were alive at the end of the study.

No tumors occurred in any of the groups of rats or mice of either sex at incidences that were significantly higher in the dosed groups than in the corresponding control groups.

It is concluded that under the conditions of this bioassay, methyl parathion was not carcinogenic for F344 rats or B6C3F$_1$ mice of either sex.

Synonym: O,O-dimethyl O(4-nitrophenyl)-phosphorothioate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-158 Bioassay of (2-Chloroethyl) trimethylammonium Chloride (CCC) for Possible Carcinogenicity (CAS No. 999-81-5)

(2-Chloroethyl)trimethylammonium chloride is a plant growth regulator, or dwarfing agent, used on poinsettias and azaleas in the United States, and on several food crops, specifically cereal grains, grapes, and pears in Europe.

A bioassay of (2-chloroethyl)trimethylammonium chloride for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F$_1$ mice.

Groups of 50 rats of each sex were administered either 1,500 or 3,000 ppm of the compound for 108 weeks, and 50 mice of each sex were administered 500 or 2,000 ppm for 102 weeks. Matched controls consisted of 20 untreated and 20 untreated mice of each sex. All surviving animals were killed at the end of the period of administration of the test chemical.

Mean body weights of dosed rats and mice were lower than those of corresponding controls for part or all of the bioassay, except for the dosed male mice, whose mean body weights were essentially the same as those of the corresponding controls. Survival was not affected significantly in any of the dosed groups of rats or mice and was at least 64% in every dosed or control group of each species at the end of the bioassay. Sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors. Since there was virtually no decrease in mean body weight in dosed male mice and only a slight decrease in female mice, and since there were no other toxic signs and no dose-related mortality, the animals may have been able to tolerate higher doses.

No tumors occurred in the rats or mice of either sex at incidences that could be associated with administration of the test chemical.

It is concluded that under the conditions of this bioassay, (2-chloroethyl)trimethylammonium chloride was not carcinogenic for F344 rats or B6C3F$_1$ mice of either sex.

Synonyms: Cyclocel®; chlormequat; chlorocholine chloride; CCC

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-159 Bioassay of Phthalic Anhydride for Possible Carcinogenicity (CAS No. 85-44-9)

Phthalic anhydride is an important chemical intermediate in the plastics industry. From it are derived numerous phthalate esters that function as plasticizers in synthetic resins. Phthalic anhydride itself is used as a monomer for synthetic resins such as glyptal, the alkyd resins, and the polyester resins. Phthalic anhydride is a precursor of anthraquinone, phthalein, rhodamine, phthalocyanine, fluorescein, and xanthene dyes. Reaction of phthalic anhydride with ammonia yields phthalimide, a useful reagent in the synthesis of primary amines, the agricultural fungicide phaltan, and thalidomide. Other reactions yield phenolphthalein, benzoic acid, phthalylsulfathiazole (an intestinal antimicrobial agent), and terephthalic acid.

A bioassay of phthalic anhydride for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F$_1$ mice.

Groups of 50 rats of each sex were administered the test chemical at one of two doses, initially either 25,000 or 50,000 ppm, for 32 weeks. Because of excessive depres-

sions in the amount of body weight gained in the dosed groups, the doses for the males were then reduced to 12,500 and 25,000 ppm, respectively, and the doses for the females were reduced to 6,250 and 12,500 ppm. Administration of the test chemical at the lowered doses was continued for 72 weeks. The time-weighted average doses for the males were either 16,346 or 32,692 ppm, and those for the females were either 12,019 or 24,038 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of the period of administration of the test chemical.

Mean body weights of the high-dose male rats and of the low- and high-dose mice of each sex were lower than those of the corresponding controls; mean body weights of the low-dose male rats and of both the low- and high-dose female rats were essentially unaffected by administration of the test chemical. Depressions in the amount of body weight gained in the male and female mice were dose related throughout the bioassay. Survivals of the rats and mice were not affected by administration of the test chemical.

No tumors occurred in the rats or mice of either sex at incidences that could be clearly related to the administration of the test chemical.

It is concluded that under the conditions of this bioassay, phthalic anhydride was not carcinogenic for F344 rats or B6C3F$_1$ mice of either sex.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-160 Bioassay of 2,4,5-Trimethylaniline for Possible Carcinogenicity (CAS No. 137-17-7)

2,4,5-Trimethylaniline is a component of a mixture of aromatic amines used in the synthesis of the red dye Ponceau 3R. This dye is produced by diazotizing a mixture of amine intermediates, some of which have been identified as methyl-, dimethyl-, or trimethylanilines, and coupling them with 2-naphthol-3,6-disulfonic acid. Ponceau 3R is therefore a complex mixture containing some 1-(2,4,5-trimethylphenylazo)-2-naphthol-3,6-disulfonic acid.

Ponceau 3R has been used as a color additive in foods since 1907. It was certified as FD&C (Food Drug and Cosmetic) Red No. 1 from 1940 until 1960, at which time it was withdrawn from general use. Provisional recertification as Ext. D&C (External Drug and Cosmetic) Red No. 15 was granted shortly thereafter in 1961, but was revoked in 1968.

A bioassay of 2,4,5-trimethylaniline for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered 2,4,5-trimethylaniline at one of two doses, either 200 or 800 ppm for the rats and either 50 or 100 ppm for the mice, for 101 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of administration of the test chemical.

Mean body weights of the dosed male and female rats were generally lower than those of corresponding controls; mean body weights of the dosed mice were only slightly lower in the males than in the corresponding controls and were unaffected or affected irregularly in the females. Survival was not affected significantly when the rats or mice were administered the test chemical and was 70% or greater in all dosed or control groups. Sufficient numbers of animals were at risk for late-appearing tumors.

In the rats, hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose related in both males and females ($P \leq 0.001$), and in direct comparisons the incidences were slightly higher in the high-dose males, high-dose females, and low-dose females ($P \leq 0.004$) than in corresponding controls (males: controls 1/19; low-dose 6/50; high-dose 20/50; females: controls 0/20; low-dose 12/49, high-dose 27/50). In addition, alveolar/bronchiolar carcinomas or adenomas occurred in the female rats at incidences that were dose related ($P = 0.003$), and in a direct comparison the incidence was significantly higher in the high-dose group ($P = 0.017$) than in the corresponding control group (controls 0/20; low-dose 3/43; high-dose 11/50).

In the female mice, hepatocellular carcinomas occurred at incidences that were dose related ($P \leq 0.001$), and in direct comparisons the incidences were significantly higher ($P \leq 0.001$) in the low- and high-dose animals than in the corresponding controls (controls 0/20, low-dose 18/49, high-dose 40/50). Because historical records of this laboratory for control B6C3F$_1$ male mice show a relatively high incidence of hepatocellular carcinomas, an increased incidence of these tumors in 2,4,5-trimethylaniline dosed male mice as compared with matched controls could not be clearly associated with administration of the test compound.

It is concluded that under the conditions of this bioassay, 2,4,5-trimethylaniline was carcinogenic for male and female F344 rats and female B6C3F$_1$ mice, inducing hepatocellular carcinomas or neoplastic nodules in the rats of each sex, alveolar/bronchiolar carcinomas in the female rats, and hepatocellular carcinomas in female mice.

Synonym: pseudocumidine

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Equivocal
Female Mice:	Positive

TR-161 Bioassay of Phthalamide for Possible Carcinogenicity (CAS No. 88-96-0)

Phthalamide is recommended for use as an accelerator for curing epoxy resins. It is believed to be used chiefly in the paint industry.

A bioassay of phthalamide for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered phthalamide at one of two doses, either 15,000 or 30,000 ppm for the males and either 5,000 or 10,000 ppm for the females, for 106 weeks. Groups of 50 mice of each sex were administered the test chemical at one of two doses, 25,000 or 50,000 ppm for the males, and at one of three doses, 6,200, 12,500, or 25,000 ppm, for the females, for 103 or 105 weeks. Matched controls consisted of 20 untreated rats of each sex, 20 untreated male mice, and two groups of 20 untreated female mice. All surviving rats and mice were killed at the end of administration of the test chemical.

Mean body weights of the dosed groups of rats and mice were either slightly lower than those of corresponding control groups or essentially unaffected by administration of the test chemical. Also, survival was unaffected in the rats and mice except for early deaths in the high- and mid-dose groups of female mice. Survival was 66% or greater at the end of the bioassay in all dosed groups and control groups of each species and sex except for the high-dose group of female mice (36%). With the exception of the high-dose female mice, sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors.

No tumors occurred in the rats or mice of either sex at incidences that were significantly higher in the dosed groups than in the corresponding control groups. However, phthalamide produced toxic lesions in the livers of male and female rats and the urinary systems of female rats and mice. The presence of nonneoplastic lesions suggests that the MTD may have been used or exceeded.

It is concluded that under the conditions of this bioassay, phthalamide was not carcinogenic for F344 rats or $B6C3F_1$ mice of either sex.

Synonyms: o-phthalic acid diamide; P-D

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-162 Bioassay of 2,4-Diaminotoluene for Possible Carcinogenicity (CAS No. 95-80-7)

2,4-Diaminotoluene is a widely used industrial intermediate. Most of this chemical produced in the United States is converted to toluene diisocyanate for use in the synthesis of polyurethanes. 2,4-Diaminotoluene is also an intermediate in the for the synthesis of dyes and used for textiles, fur, leather, biological stains and indicators, spirit varnishes and wood stains, and pigments. In addition, it has been used as a component of oxidation-type hair formulations. Two hundred and thirty-three million pounds of 2,4-diaminotoluene were produced in the United States in 1976. In addition, 356,000 pounds were imported in that year.

A bioassay of 2,4-diaminotoluene for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered 2,4-diaminotoluene at one of two doses, initially either 125 or 250 ppm, for 40 weeks. Because of excessive depression in the amount of mean body weight gained in both low- and high-dose groups, doses were then reduced to 50 and 100 ppm, respectively. Administration of 50 ppm to the low-dose groups was continued for 63 weeks, and surviving animals in these groups were then killed. Surviving animals in the high-dose males and females administered 100 ppm were killed at the end of 39 and 44 weeks, respectively, due to morbidity. The time-weighted average dose was 79 ppm for the low-dose male and females for 103 weeks, 176 ppm for the high-dose males for 79 weeks, and 171 ppm for the high-dose females for 84 weeks. Matched controls consisted of 20 untreated rats of each sex.

Groups of 50 mice of each sex were administered 2,4-diaminotoluene at one of two doses, either 100 or 200 ppm, for 101 weeks. Matched controls consisted of 20 untreated mice of each sex. Surviving mice were killed at the end of administration of the test chemical.

Mean body weights of dosed male and female rats and mice were lower than those of corresponding controls and were dose related except for the low-dose male mice, for which mean body weights were only slightly lower than those of controls. Mortality was not dose related in either the male or female mice, but was dose related in both the male and female rats. Survival was decreased and lesions of hepatonephrotoxicity were observed in the animals administered the 2,4-diaminotoluene.

In the rats, hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose related in both the males ($P = 0.014$) and the females ($P = 0.008$). In direct comparisons of incidences of the tumors in control and dosed groups, the incidence in the high-dose male group had a P value of 0.026 (males: controls 0/20, low-dose 5/49, high-dose 10/50; females: controls 0/20; low-dose 0/50, high-dose 6/49). The significance of the occurrence of these tumors in both the male and female rats was supported by the high incidences of associated nonneoplastic lesions of the liver in the dosed groups and by low incidences of liver tumors in historical-control male or female F344 rats at the same laboratory.

In addition, carcinomas or adenomas of the mammary gland occurred in the female rats at incidences that were dose related ($P = 0.002$) and in direct comparisons were

higher in the dosed groups ($P<0.001$) than in the control group (control 1/20; low-dose 38/50, high-dose 41/50).

In male rats, fibromas of the subcutaneous tissue occurred at incidences that were dose related ($P=0.004$) and in direct comparisons were higher in the dosed groups ($P\leq 0.020$) than in the control group (controls 0/20, low-dose 15/30, high-dose 19/50).

In the mice, hepatocellular carcinomas occurred in the females at incidences that were dose related ($P=0.002$) and in direct comparisons were higher in dosed groups ($P\leq 0.007$) than in the control group (controls 0/19, low-dose 13/47, high-dose 18/46). In addition, lymphomas occurred at a significant incidence ($P<0.001$) in the low-dose female mice (controls 2/19, low-dose 29/47, high-dose 11/46). No tumors occurred at significantly increased incidences in the dosed male mice.

Under the conditions of this bioassay, 2,4-diaminotoluene was carcinogenic for F344 rats, inducing hepatocellular carcinomas or neoplastic nodules in both males and females and carcinomas or adenomas of the mammary gland in females. The test chemical was also carcinogenic for female $B6C3F_1$ mice, inducing hepatocellular carcinomas. The incidence of lymphomas in the female mice suggested that these tumors may also have been related to administration of the test chemical.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Positive

TR-163 Bioassay of Calcium Cyanamide for Possible Carcinogenicity (CAS No. 156-62-7)

Calcium cyanamide was first synthesized in 1898 and became one of the earliest successes in nitrogen fixation. The commercially formulated product contains approximately 65% calcium cyanamide, which is 20 to 24% nitrogen. For most of the 20th century it has been used as a fertilizer, and also as a cotton defoliant, herbicide, and soil insecticide. Its use as a fertilizer has diminished in recent years due to the introduction of other compounds, so that the chief industrial uses of calcium cyanamide today stem from the reactivity of the nitrile group. Calcium cyanamide can be dimerized to dicyandiamide, an intermediate for melamine, one of the basic ingredients in amino plastics and resins. Other products prepared from calcium cyanamide include urea, thiourea, and guanidine. Fusion of calcium cyanamide with sodium chloride produces calcium cyanide, which is required for ore processing and the production of ferrocyanides. Calcium cyanamide is added to pig iron to impart nitrogen and to remove sulfur from steel.

A bioassay of formulated calcium cyanamide for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered a commercial formulation containing 63% calcium cyanamide in the diet at one of two doses, either 100 or 200 ppm for the males and either 100 or 400 ppm for the females, for 107 weeks. Groups of 50 mice of each sex were administered the test chemical at one of two doses, either 500 or 2,000 ppm, for 100 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of administration of the test chemical.

Mean body weights of the dosed rats and mice were only slightly lower than those of corresponding controls, except for the low-dose female mice, whose mean body weights were unaffected by the test chemical. Mortality was dose related only in male mice. Survival was 70% or greater in all dosed and control groups of each species and sex at the end of the bioassay, and sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors. Both rats and mice may have been able to tolerate higher doses.

No tumors occurred in the dosed rats of either sex at incidences that could clearly be related to administration of the calcium cyanamide. However, in the subchronic studies performed with the rats, calcium cyanamide was found to cause diffuse follicular hyperplasia of the thyroid, with periglandular fibrosis and prominent periglandular vascularity.

In male mice, hemangiosarcomas were dose related in the males ($P=0.006$); however, in direct comparisons, incidences in the individual dosed groups were not significantly higher than those in the control group (controls 1/20 (5%); low-dose 2/50 (4%); high-dose 10/50 (20%)). The incidence of these tumors in historical-control male $B6C3F_1$ mice was (13/323 (4%)), and the highest incidence observed was 2/19 (10%). In female mice, lymphomas or leukemias were dose related ($P=0.009$), and in a direct comparison the incidence of these tumors in the high-dose group was significantly higher ($P=0.006$) than that in the control group (controls 1/20 (5%); low-dose 11/46 (24%); high-dose 18/50 (36%)); however, the incidence of the lymphomas or leukemias in historical-control female $B6C3F_1$ mice was 67/324 (21%), suggesting that the incidence of these tumors in the matched-control group of the present bioassay may have been abnormally low. Thus, neither the incidences of hemangiosarcomas of the circulatory system in male mice nor of lymphomas or leukemias in the female mice can clearly be related to administration of the test chemical.

It is concluded that under the conditions of this bioassay, the test formulation of calcium cyanamide was not carcinogenic for F344 rats or $B6C3F_1$ mice of either sex.

Synonyms: cyanamide; CaNCN

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-164 Bioassay of N-Nitrosodiphenylamine for Possible Carcinogenicity (CAS No. 86-30-6)

N-Nitrosodiphenylamine is a nitrosoamine which is used as a vulcanization retarder in curing natural rubber and the synthetic elastomers styrenebutadiene and nitrile-butadiene. U.S. production in 1976 was 1.3 million pounds.

A bioassay of N-nitrosodiphenylamine for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered N-nitrosodiphenylamine at one of two doses, either 1,000 or 4,000 ppm, for 100 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at the end of administration of the test chemical.

Groups of 50 male mice were administered N-nitrosodiphenylamine at one of two doses, either 10,000 or 20,000 ppm, for 101 weeks. Groups of 50 female mice were administered the test chemical at one of two doses, initially 5,000 or 10,000 ppm, for 38 weeks. Because of excessive depression in the amount of mean body weight gained in the dosed groups, the doses for the females were then reduced to 1,000 and 4,000 ppm, respectively, and administration at the lowered doses was continued for 60 weeks. The time-weighted average doses for the female mice were either 2,315 or 5,741 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of administration of the test chemical.

Mean body weights of dosed rats and mice of each sex were lower than those of corresponding controls, and were dose related throughout the bioassay, except for those of female rats during the first part of the bioassay. Mortality was dose related in the female rats, but was not affected when the test chemical was administered to the male rats or the male or female mice. Survival at the end of the bioassay was 64% or greater in the dosed and control groups of rats and mice of each sex, and sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors.

Transitional-cell carcinomas of the urinary bladder occurred at incidences that were dose related ($P \leq 0.001$) in both male and female rats, and in direct comparisons the incidences of these tumors in the high-dose groups of each sex were significantly higher ($P \leq 0.001$) than those in the corresponding controls (males: controls 0/19; low-dose 0/46; high-dose 16/45; females: controls 0/18; low-dose 0/48; high-dose 40/49). The possible mechanism by which these tumors were induced, such as calculi formation in the bladder or nitrosation of amines present in feed to a carcinogenic nitrosoamine, is unknown.

Fibromas of the integumentary system occurred in male rats at incidences that were dose related ($P = 0.003$), although in direct comparisons the incidences of these tumors in the individual dosed groups were not significantly higher than those in the control group (controls 1/20, or 5%; low-dose 1/50, or 2%; high-dose 10/50, or 20%). The incidence of fibromas of the integumentary system in historical-control male F344 rats at this laboratory is 6/285, or 2%. These results suggest an association of the fibromas in the male rats with the administration of the test chemical.

No tumors occurred in the mice of either sex at incidences that were significantly higher in the dosed groups than in the corresponding control groups. The only changes related to compound administration were chronic inflammatory lesions in the urinary bladders of dosed mice.

It is concluded that under the conditions of this bioassay, N-nitrosodiphenylamine was carcinogenic for both sexes of F344 rats, including transitional-cell carcinomas of the urinary bladder, but was not carcinogenic for $B6C3F_1$ mice of either sex.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-165 Bioassay of 4-Chloro-o-toluidine Hydrochloride for Possible Carcinogenicity (CAS No. 3156-93-3)

4-Chloro-o-toluidine hydrochloride is used commercially as an intermediate for dyestuffs intended for textiles, e.g., pigment yellow 49 and pigment red 7, as well as C.I, 12800, azoic coupling component 8, and azoic diazo component 11.

A bioassay of 4-chloro-o-toluidine hydrochloride for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered 4-chloro-o-toluidine in the diet at one of two doses, either 1,250 or 5,000 ppm, for 107 weeks. Groups of 50 mice of each sex were administered the test chemical in the diet at one of two doses, either 3,750 or 15,000 ppm for the males and either 1,250 or 5,000 ppm for females, for 99 weeks, except for the high dose females (92 weeks). Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of administration of the test chemical.

Mean body weights of the high-dose rats and the low- and high-dose mice of each sex were lower than those of corresponding controls, and those of the mice were dose related. Mortality was not significantly affected by administration of the test chemical to rats of either sex

and survival was 75% or greater at the end of the study in dosed and control groups. Sufficient numbers of rats were at risk for the development of late-appearing tumors. In mice, mortality was dose related for each sex.

In rats no tumors occurred at incidences which could clearly be related to administration of the test chemical.

In both male and female mice, hemangiosarcomas occurred at incidences that were dose related ($P \leq 0.001$), and in direct comparisons the incidences in the high-dose males and the low- and high-dose females were significantly higher ($P < 0.001$) than those in the corresponding controls (males: controls 0/20; low-dose 3/50; high-dose 37/50; females: controls 0/18; low-dose 40/49, high-dose 39/50). The combined incidences of hemangiosarcomas and hemangiomas also were dose related and were significantly higher in the dosed groups of male and female mice than in the corresponding controls. There was a high incidence of hemosiderin deposit in the renal tubular epithelium, particularly in mice with hemangiosarcomas.

It is concluded that under the conditions of this bioassay, 4-chloro-o-toluidine hydrochloride was not carcinogenic for F344 rats but was carcinogenic for $B6C3F_1$ mice, including hemangiosarcomas and hemangiomas in both males and females.

Synonym: 2-amino-4-chlorotoluene hydrochloride

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-166 Bioassay of Tetraethylthiuram Disulfide for Possible Carcinogenicity (CAS No. 97-77-9)

Tetraethylthiuram disulfide is known in the rubber industry as ethyl tuads where it is used in compounding natural rubber and the synthetic elastomers isobutylene-isoprene, butadiene, styrene-butadiene, isoprene, and nitrile-butadiene rubber. It is used both as a rubber accelerator and vulcanizing agent, as an activator of thiazole accelerators, and as a plasticizer in neoprene. Current estimates indicate that 510,000 to 550,000 kilograms of chemical are produced annually worldwide.

A bioassay of technical-grade tetraethylthiuram disulfide for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered tetraethylthiuram disulfide in the diet at one of two doses, either 300 or 600 ppm, for 107 weeks. Groups of 50 mice of each sex were administered the test chemical at one of two doses, either 500 or 2,000 ppm for the males and either 100 or 500 ppm for the females, for 108 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the ends of the periods of administration of the test chemical.

Mean body weights of the dosed rats and mice of each sex were lower than those of corresponding controls and were dose related throughout most of the bioassay. Mortality was not significantly affected by administration of the test chemical to either the rats of the mice, except for the female rats, in which the mortality was higher in the control group than in the dosed groups; however, the survival at the end of the bioassay was 65% or greater in all dosed and control groups of rats and mice of either sex, and sufficient numbers of animals were at risk in each group for the development of late-appearing tumors.

No tumors occurred in the rats or mice of either sex at incidences that were significantly higher in dosed groups than in corresponding control groups.

It is concluded that under the conditions of this bioassay, tetraethylthiuram disulfide was not carcinogenic for F344 rats or $B6C3F_1$ mice of either sex.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-167 N-Butylurea (CAS: 592-31-4) Sodium Nitrite (CAS: 7632-00-0)

Data considered to be inconclusive and not reportable; no Technical Report issued.

TR-168 Bioassay of N-(1-Naphthyl) ethylenediamine Dihydrochloride for Possible Carcinogenicity (CAS No. 1465-25-4)

N-(1-Naphthyl)ethylenediamine dihydrochloride, a diagnostic reagent derived from 1-naphthylamine, was selected for bioassay by the National Cancer Institute because of the suspected carcinogenicity of its parent compound, and the confirmed bladder carcinogenicity of the related compound 2-naphthylamine in humans.

A bioassay for the possible carcinogenicity of N-(1-naphthyl)ethylenediamine dihydrochloride was conducted using Fischer 344 rats and $B6C3F_1$ mice. N-(1-Naphthyl)ethylenediamine dihydrochloride was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty-five rats of each sex and 50 mice of each sex were placed on test as controls. The high and low dietary concentrations of N-(1-naphthyl)ethylenediamine dihydrochloride administered to rats and male mice were 0.1 and 0.05 percent, respectively. The high and low time-

weighted average concentrations administered to female mice were, respectively, 0.3 and 0.2 percent. The compound was administered in the diet for 104 weeks, followed by an observation period of 4 weeks for high dose rats, 3 weeks for low dose rats, low dose female mice, and high dose female mice, and 1 week for high dose male mice.

There were no significant positive associations between the concentrations of N-(1-naphthyl) ethylenediamine dihydrochloride administered and mortality in rats of either sex or in male mice. There was a significant positive association between concentration and mortality in female mice. In all groups, except for high dose females, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression, in relation to controls, was apparent for both sexes of rats and mice, indicating that higher concentrations of the test chemical would not have been tolerated by these animals.

In rats or mice of either sex, there were no statistically significant positive associations between the concentration of N-(1-naphthyl)ethylenediamine dihydrochloride and tumor incidence.

Under the conditions of this bioassay, dietary administration of N-(1-naphthyl)ethylenediamine dihydrochloride was not carcinogenic in Fischer 344 rats or $B6C3F_1$ mice.

Synonyms: N-1-Naphthalenyl-1,2-ethanediamine dihydrochloride; N-1-Naphthylethylenediamine dihydrochloride

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-169 Bioassay of 2-Nitro-p-phenylenediamine for Possible Carcinogenicity (CAS No. 5307-14-2)

2-Nitro-p-phenylenediamine. a component of both semipermanent and permanent hair dye formulations, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer among dye manufacturing industry workers. Aromatic amines are one of several classes of organic chemicals thought to contribute to the increased cancer risk in this industry. The widespread exposure to 2-nitro-p-phenylenediamine among the general population, and the possibility of an increased cancer risk among hairdressers were additional factors in the selection of this compound for testing.

A bioassay for the possible carcinogenicity of 2-nitro-p-phenylenediamine was conducted using Fischer 344 rats and $B6C3F_1$ mice. 2-Nitro-p-phenylenediamine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 2-nitro-p-phenylenediamine were, respectively, 1,100 and 550 ppm for male rats, 2,200 and 1,100 ppm for female rats, and 4,400 and 2,200 ppm for mice of both sexes. The compound was administered in the diet for 78 weeks, followed by an observation period of 27 weeks for rats and 12 to 13 weeks for mice.

There were no significant positive associations between the dietary concentrations of 2-nitro-p-phenylenediamine administered and mortality in rats and mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression, relative to controls, was observed in dosed rats and mice of both sexes, indicating that the concentrations administered to these animals may have approximated the maximum tolerated dosages.

When the female mice in each group, having hepatocellular carcinoma or hepatocellular adenoma, were combined and the resulting incidences statistically analyzed, there was a significant positive association between concentration administered and the incidence of these tumors. This finding was supported by a significant high dose to control Fischer exact comparison. No tumors occurred in statistically significant increased incidences when dosed male or female rats or male mice were compared to their respective controls.

Under the conditions of this bioassay, dietary administration of 2-nitro-p-phenylenediamine was carcinogenic to female $B6C3F_1$ mice, causing an increased incidence of hepatocellular neoplasms, primarily hepatocellular adenomas. There was no convincing evidence for the carcinogenicity of the compound in Fischer 344 rats or in male $B6C3F_1$ mice.

Synonyms: 2-Nitro-1,4-benzenediamine; Diaminonitrobenzene; m-Nitro-p-phenylenediamine; o-Nitro-p-phenylenediamine; 2-Nitro-1,4-diaminobenzene; 1,4-Diamino-2-nitrobenzene; 2-NP; 2-NPPD; 2-N-p-PDA; Ursol Brown RR; Zoba Brown RR; Fourrine Brown 2R; Fourrine 36; Fouramine 2R; C.I. Oxidation Base 22

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Positive

TR-170 Bioassay of a Solution of β-Nitrostyrene and Styrene for Possible Carcinogenicity (CAS No. 102-96-5, CAS No. 100-42-5)

β-Nitrostyrene was selected for bioassay by the National Cancer Institute because of a lack of adequate

carcinogenicity data. The compound is usually supplied as a 30 percent solution in styrene and this commercial product was selected as the material to be tested.

β-Nitrostyrene is used as a chain stopper in styrene type polymerization reactions for the production of polystyrene plastics, synthetic rubber, and resins. β-Nitrostyrene also possesses antibacterial, antifungal, and insecticidal activities and has been suggested for use as a repellent for bats and other rodents; however, this compound does not appear to be currently registered for pesticide use with the U.S. Environmental Protection Agency.

A bioassay of a solution of 30 percent β-nitrostyrene and 70 percent styrene for possible carcinogenicity was conducted using Fischer 344 rats and $B6C3F_1$ mice. The solution of the two test materials in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The high and low dosages utilized in the study were, respectively, 300 and 150 mg/kg for male rats; 150 and 75 mg/kg for female rats; and 175 and 87.5 mg/kg for mice of both sexes. These dosages are expressed in terms of the β-nitrostyrene contained in the styrene solution. Twenty animals of each species and sex were placed on test as controls, and were gavaged with corn oil on the same schedule as dosed animals.

A 79-week period of chemical administration was followed by an additional observation period of 29 weeks for rats, and a 78-week period of chemical administration was followed by an additional 14-week observation period for mice.

There was no significant difference between the survival of rats dosed with the test solution and that of their controls, and there was no significant association between dosage and mortality among female mice. There was a significant positive association between dosage and mortality among male mice; however, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. There was distinct mean body weight depression when high dose female mice or male rats were compared to their controls, indicating that the dosage administered to these animals may have approximated the maximum tolerated dosage. Since no distinct mean body weight depression, no significantly accelerated mortality, and no other toxic effects were associated with the administration of β-nitrostyrene and styrene to female rats or male mice, it is possible that these animals may have been able to tolerate a higher dosage.

There were no significant positive associations between administration of the solution and increased tumor incidence in rats of either sex.

When those male mice having either alveolar/bronchiolar carcinomas or alveolar/bronchiolar adenoma were combined and the resulting tumor incidences for each group were statistically analyzed, the low dose to control Fischer exact comparison was significant. The Cochran-Armitage test and the high dose to control comparison, however, were not. No other tests for tumors of any site in either male or female mice were significant.

Under the conditions of this bioassay, there was no convincing evidence for the carcinogenicity of a solution of β-nitrostyrene and styrene in Fischer 344 rats or in $B6C3F_1$ mice.

Synonyms for β-Nitrostyrene: (2-Nitroethenyl)benzene; ω-Nitrostyrene; BNS

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-171 Bioassay of 2,4-Dimethoxyaniline Hydrochloride for Possible Carcinogenicity (CAS No. 54150-69-5)

2,4-Dimethoxyaniline hydrochloride, the hydrochloride salt of the dye intermediate 2,4-dimethoxyaniline, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer among dye manufacturing industry workers. Aromatic amines are one of several classes of chemicals thought to contribute to the increased cancer risk in this industry.

A bioassay for the possible carcinogenicity of 2,4-dimethoxyaniline HCl was conducted using Fischer 344 rats and $B6C3F_1$ mice. 2,4-Dimethoxyaniline HCl was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 2,4-dimethoxyaniline HCl were, respectively, 3,000 and 1,500 ppm for rats and 5,000 and 2,500 ppm for mice. The compound was administered in the diet for 104 weeks to rats and 103 weeks to mice, followed by a 1-week observation period for both species.

There were no significant positive associations between the concentrations of 2,4-dimethoxyaniline HCl administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Dose-related mean body weight depression was observed for females of both species, indicating that the concentrations of 2,4-dimethoxyaniline HCl administered to these groups may have approximated the maximum tolerated concentrations. Compound-related mean body weight depression was only slight for male rats and was apparent in male mice only until week 50; however follicular-cell hyperplasias and cystic follicles of the thyroid were observed in dosed male mice, suggesting that the concentrations the male mice received may have approximated the maximum tolerated concentrations. Since no distinct mean body weight depression in relation to controls, no significant accelerated mortality, and no other signs of toxicity were associated with

administration of 2,4-dimethoxyaniline HCl to male rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

There was a significant positive trend between concentration of the test chemical and the incidence of a combination of hepatocellular carcinomas and adenomas in male mice and an increase in the combination of these lesions in female mice. However, no statistically significant differences in tumor incidence at any site were observed when dosed rats and mice were compared to their respective controls.

Under the conditions of this bioassay there was no convincing evidence for the carcinogenicity of 2,4-dimethoxyaniline HCl in Fischer 344 rats or $B6C3F_1$ mice.

Synonyms: 2,4-dimethoxybenzenamine hydrochloride; 4-methoxy-o-anisidine hydrochloride; 2-methoxy-p-anisidine hydrochloride

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-172 Bioassay of Sodium Diethyldithiocarbamate for Possible Carcinogenicity (CAS No. 148-18-5)

Sodium diethyldithiocarbamate is a chelating agent used primarily in the analytical determination of copper, arsenic, nickel, and other metals. Other applications include the detection of toxic metals in urine, and in the treatment of human poisoning with metals.

A bioassay of sodium diethyldithiocarbamate for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were administered sodium diethyldithiocarbamate at one of two doses, either 1,250 or 2,500 ppm, for 104 weeks. Groups of 50 mice of each sex were administered sodium diethyldithiocarbamate at one of two doses, either 500 or 4,000 ppm, for 108 or 109 weeks. Matched controls consisted of 16 untreated male rats, 20 untreated female rats and 20 untreated mice of each sex. All surviving rats and mice were killed at the end of administration of the test chemical.

Mean body weights of all dosed groups of rats and mice were lower that those of corresponding controls and were dose related throughout the bioassay except those of the low-dose male rats, which were essentially unaffected by administration of the test chemical. Survivals of the rat and mice were unaffected, and no other clinical signs could be related to administration of the test chemical; thus, the animals may have been able to tolerate higher doses. Sufficient numbers of dosed and control animals of each species and sex were at risk for the development of late-appearing tumors.

No tumors occurred in the rats or mice of either sex at incidences that were significantly higher in the dosed groups than in the control groups.

It is concluded that under the conditions of this bioassay, sodium diethyldithiocarbamate was not carcinogenic for F344 rats or $B6C3F_1$ mice of either sex.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-173 Bioassay of Carbromal for Possible Carcinogenicity (CAS No. 77-65-6)

Carbromal, a mild central nervous system depressant, was selected for bioassay by the National Cancer Institute because of the similarity of the biological activity of this compound to that of urethan, which is known to induce leukemia in mice and is an initiator of skin carcinogenesis in mice, and the widespread exposure to this compound among the general population via deliberate ingestion for medicinal purposes.

A bioassay for the possible carcinogenicity of carbromal was conducted using Fischer 344 rats and $B6C3F_1$ mice. Carbromal was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species with the exception of 49 low dose male mice and high dose female mice. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of carbromal were, respectively, 2,500 and 1,250 ppm for rats and 2,500 and 1,250 ppm for mice. The compound was administered for 103 weeks to rats and for 78 weeks to mice. The period of compound administration was followed by an observation period of 1 week for rats and 26 weeks for mice.

There was no significant positive associations between the concentrations of carbromal administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Slight dose-related mean body weight depression was observed for male rats and for females of both species and the mean body weight among dosed male mice was lower than that for controls, indicating that the concentrations of carbromal administered to the animals in this bioassay may have approximated the maximum tolerated concentrations.

None of the statistical tests for any site in female rats or in mice of either sex indicated a significant positive association between compound administration and tumor incidence. There was a significant positive association between the concentrations administered and the incidences of adrenal pheochromocytomas in male rats; however, the Fischer exact comparisons were not significant.

Under the conditions of this bioassay, dietary administration of carbromal was not carcinogenic in Fischer 344 rats or B6C3F$_1$ mice.

Synonyms: N-(aminocarbonyl)-2-bromo-2-ethyl-butanamide; (2-bromo-2-ethylbutyryl)urea, bromodiethylacetylcarbamide; bromodiethylacetylurea; (α-bromo-α-ethylbutyryl)carbamide

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-174 Bioassay of p-Phenylenediamine Dihydrochloride for Possible Carcinogenicity (CAS No. 624-18-0)

p-Phenylenediamine dihydrochloride, a hydrochloride salt of p-phenylenediamine, the major component of many oxidation hair dyes, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer reported among dye manufacturing industry workers. Aromatic amines are one of several classes of chemicals thought to contribute to this increased cancer risk. The widespread exposure to p-phenylenediamine among the general population and the increased cancer risk among hairdressers were additional factors in the selection of p-phenylenediamine dihydrochloride for testing.

A bioassay of p-phenylenediamine dihydrochloride for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F$_1$ mice. p-Phenylenediamine dihydrochloride was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low concentrations of p-phenylenediamine dihydrochloride were, respectively, 1,250 and 625 ppm for both rats and mice. After a 103-week period of compound administration, there were additional observation periods of 2 weeks for rats and 1 week for mice. Twenty animals of each sex and species were placed on test as controls.

There were no significant positive associations between the concentrations of p-phenylenediamine dihydrochloride administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late developing tumors. Slight dose-related mean body weight depression was observed in female rats and the mean body weights among high dose male rats and dosed female mice were slightly depressed in relation to their respective controls, indicating that the concentrations of p-phenylenediamine dihydrochloride administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no distinct mean body weight depression relative to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of p-phenylenediamine dihydrochloride to male mice, it is possible that these animals may have been able to tolerate a higher dietary concentration.

None of the statistical tests for any site in rats or mice of either sex, including time to leukemia or malignant lymphoma analysis in female mice, indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, there was no convincing evidence that dietary administration of p-phenylenediamine dihydrochloride was carcinogenic in Fischer 344 rats or B6C3F$_1$ mice.

Synonyms: 1,4-benzenediamine dihydrochloride; p-PDA HCl; p-OD HCl; p-phenylenediamine di-HCl; Durafur Black RC; Fourrine DS; Fourrine 64; Pelagol Grey CD; C.I. Oxidation Base 10A

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-175 Bioassay of Lithocholic Acid for Possible Carcinogenicity (CAS No. 434-13-9)

Lithocholic acid, a naturally occurring bile acid, was selected for bioassay by the National Cancer Institute because it has been reported to promote the development of hepatoma and hyperplastic nodules induced by DL-ethionine in rat liver, and because of the strong correlation between concentrations of neutral sterols and bile acid derivatives in human feces and the incidence of human colon cancer.

A bioassay for the possible carcinogenicity of lithocholic acid was conducted using Fischer 344 rats and B6C3F$_1$ mice. Lithocholic acid was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species, except for 49 low dose female rats. Twenty animals of each sex and species were placed on test as controls. The high and low dosages of lithocholic acid administered were, respectively, 500 and 250 mg/kg for rats and 250 and 125 mg/kg for mice. The compound was administered to rats and mice for 103 weeks. The period of compound administration was followed by an observation period of 1 week for rats and 2 weeks for mice.

There were no significant positive associations between the dosages of lithocholic acid administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Slight dose-related mean body weight depression was observed in male rats

and female mice and high incidences of chronic kidney inflammation were observed in female rats, indicating that the dosages of lithocholic acid administered to these animals in this bioassay may have approximated the maximum tolerated dosages. Since no mean body weight depression, relative to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of lithocholic acid to male mice, it is possible that these animals may have been able to tolerate a higher dosage. However, in the subchronic study there were deaths among all dosed male mouse groups, even those receiving lithocholic acid at a level only twofold greater than the high dose utilized in the chronic study.

None of the statistical tests for any site in rats or in mice of either sex indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, lithocholic acid was not carcinogenic when administered by gavage to Fischer 344 rats or B6C3F$_1$ mice.

Synonyms: (3α,5β)-3-Hydroxycholan-24-oic acid; 3α-Hydroxy-5β-cholan-24-oic acid; 3α-Hydroxy-5β-cholanic acid; 3α-Hydroxychloanic acid; 3-Monohydroxycholanic acid; 17β-(1-methyl-3-carboxypropyl)ethiocholan-3α-ol.

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-176 N,N-Dimethyl-P-Nitrosoaniline (CAS: 138-89-6)

Data considered to be inconclusive and not reportable; no Technical Report issued.

TR-177 Bioassay of 4'-(Chloroacetyl)-acetanilide for Possible Carcinogenicity (CAS No. 140-49-8)

4'-(Chloroacetyl)-acetanilide, an intermediate in the synthesis of dyes and pharmaceutical compounds, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer observed among dye manufacturing industry workers. Aromatic amines, such as 4'-(chloroacetyl)-acetanilide, are among several classes of chemicals thought to contribute to the increased cancer risk in this industry, and 4'-(chloroacetyl)-acetanilide is especially suspect because it is structurally similar to the possible human renal pelvic carcinogen, phenacetin.

A bioassay for the possible carcinogenicity of 4'-(chloroacetyl)-acetanilide was conducted using Fischer 344 rats and B6C3F$_1$ mice. 4'-(Chloroacetyl)-acetanilide was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 4'-(chloroacetyl)-acetanilide were, respectively, 2,000 and 1,000 ppm for rats and 10,000 and 5,000 ppm for mice. The compound was administered for 87 weeks of a 102-week period in rats and for 90 weeks of a 105-week period in mice. Mice were killed at the end of the last week of compound administration, while rats were observed for 1 week after compound administration ceased.

There were no significant positive associations between the concentration of 4'-(chloroacetyl)-acetanilide administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Dose-related mean body weight depression was observed for males and females of both species, indicating that the concentrations of 4'-(chloroacetyl)-acetanilide administered to the animals in this bioassay may have approximated the maximum tolerated concentrations.

None of the statistical tests for any site in rats of either sex or in male mice indicated a significant positive association between compound administration and tumor incidence. Although there was a significant positive association between the concentration of the compound administered and the incidences of hepatocellular adenomas in female mice, the Fischer exact comparisons were not significant.

Under the conditions of this bioassay, 4'-(chloroacetyl)-acetanilide was not carcinogenic when administered in the diet to Fischer 344 rats or B6C3F$_1$ mice of either sex.

Synonyms: N'-(Chloroacetyl)-N-phenylacetamide; 4-(Cl-acetyl)acetanilide

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-178 Bioassay of 2-(Chloromethyl) pyridine Hydrochloride for Possible Carcinogenicity (CAS No. 6959-47-3)

2-(Chloromethyl)pyridine hydrochloride, an aromatic heterocycle used in a variety of syntheses, was selected for bioassay by the National Cancer Institute because of the structural similarity of this compound to 2-(α,β-dichloroethyl)-pyridine hydrochloride, a carcinogen in rats, mice, Syrian hamsters, and Mongolian gerbils.

A bioassay for the possible carcinogenicity of 2-(chloromethyl)pyridine hydrochloride was conducted using Fischer 344 rats and $B6C3F_1$ mice. 2-(Chloromethyl)pyridine hydrochloride was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species, with the exception of 49 male rats in the high dose group. Twenty animals of each sex and species were placed on test as vehicle controls. The high and low dosages of 2-(chloromethyl)pyridine hydrochloride administered were, respectively, 150 and 75 mg/kg for rats and 250 and 125 mg/kg for mice. The compound was administered for 99 weeks to rats and mice. The period of compound administration was followed by an observation period of 6 weeks for rats and 5 weeks for mice.

There were no significant positive associations between the dosages of 2-(chloromethyl)pyridine hydrochloride administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Slight dose-related mean body weight depression was observed in mice of both sexes, indicating that the dosages of 2-(chloromethyl)pyridine hydrochloride administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no distinct mean body weight depression relative to vehicle controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of 2-(chloromethyl) pyridine hydrochloride to rats, it is possible that these animals may have been able to tolerate a higher dosage.

None of the statistical tests for any site in female rats or in mice of either sex indicated a significant positive association between compound administration and tumor incidence. There was a significant positive trend between the dosages administered and the incidences of subcutaneous fibromas in male rats. The Fischer exact comparisons, however, were not significant.

Under the conditions of this bioassay, administration of 2-(chloromethyl)pyridine hydrochloride was not carcinogenic to Fischer 344 rats or $B6C3F_1$ mice.

Synonyms: 2-(Cl-methyl)pyridine HCl; 2-pyridylmethyl chloride hydrochloride; 2-picolyl chloride hydrochloride

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-179 Bioassay of p-Quinone Dioxime for Possible Carcinogenicity (CAS No. 105-11-3)

p-Quinone dioxime, a rubber vulcanization accelerator, was selected for bioassay by the National Cancer Institute because of a lack of adequate carcinogenicity data.

A bioassay for the possible carcinogenicity of p-quinone dioxime was conducted using Fischer 344 rats and $B6C3F_1$ mice. p-Quinone dioxime was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls, with the exception of 18 in the control male mouse group. The high and low concentrations of p-quinone dioxime were 750 and 375 ppm for rats and 1,500 and 750 ppm for mice. The compound was administered to rats and mice for 104 weeks. The period of compound administration was followed by an observation period of 1 week for both species.

There were no significant positive associations between the concentrations of p-quinone dioxime administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Distinct dose-related mean body weight depression was observed among rats and slight mean body weight depression, relative to controls, was observed among mice, indicating that the dosages of p-quinone dioxime administered to the animals in this bioassay may have approximated the maximum tolerated concentrations.

Tumors of the urinary bladder were observed only in dosed rats. For female rats, there was a significant positive association between concentration administered and the incidences of a combination of urinary bladder neoplasms. The high dose to control Fischer exact comparison was also significant for these tumors in female rats. No compound-related neoplasms were observed in male rats or mice of either sex.

Under the conditions of this bioassay, dietary administration of p-quinone dioxime was carcinogenic to female Fischer 344 rats, causing neoplasms of the urinary bladder. The compound was not carcinogenic to male Fischer 344 rats or $B6C3F_1$ mice of either sex.

Synonyms: 2,5-cyclohexadiene-1,4-dione; dioxime p-benzoquinone; p-quinonedioxime; dioxime 1,4-cyclohexadienedione

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Negative

TR-180 Bioassay of 4-Nitro-o-phenylenediamine for Possible Carcinogenicity (CAS No. 99-56-9)

4-Nitro-o-phenylenediamine, a component of both semipermanent and permanent hair dye formulations,

was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer reported among workers in the dye manufacturing industry.

A bioassay for the possible carcinogenicity of 4-nitro-o-phenylenediamine was conducted using Fischer 344 rats and B6C3F$_1$ mice. 4-Nitro-o-phenylenediamine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 4-nitro-o-phenylenediamine were, respectively, 750 and 375 ppm for rats and 7500 and 3750 ppm for mice. The compound was administered for 103 weeks to rats and for 102 weeks to mice. The period of compound administration was followed by an observation period of 2 weeks for rats and mice.

There were no significant positive associations between the concentrations of 4-nitro-o-phenylenediamine administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Distinct dose-related mean body weight depression was observed in mice, indicating that the concentrations of 4-nitro-o-phenylenediamine administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no distinct mean body weight depression relative to controls, no significantly accelerated mortality, and no other manifestations of chronic toxicity were associated with administration of 4-nitro-o-phenylenediamine to male or female rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

None of the statistical tests for any site in rats or in mice of either sex indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, dietary administration of 4-nitro-o-phenylenediamine was not carcinogenic in Fischer 344 rats or B6C3F$_1$ mice.

Synonyms: 4-nitro-1,2-benzenediamine; 4-nitrophenylenediamine; 4-nitro-1,2-diaminobenzene; 1,2-diamino-4-nitrobenzene; 2-amino-4-nitroaniline; 4-NO; 4-NOP; 4-NOPD; 4-N-o-PDA; C.I. 76020

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-181 Bioassay of Michler's Ketone for Possible Carcinogenicity (CAS No. 90-94-8)

Michler's ketone, a dye intermediate and derivative of dimethylaniline, was selected for bioassay by the National Cancer Institute because of the elevated incidence of bladder cancer noted among dye manufacturing industry workers.

A bioassay for the possible carcinogenicity of technical-grade Michler's ketone was conducted using Fischer 344 rats and B6C3F$_1$ mice. Michler's ketone was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of Michler's ketone were respectively, 500 and 250 ppm for male rats, 1,000 and 500 ppm for female rats, and 2,500 and 1,250 ppm for mice of both sexes. The compound was administered to rats and mice for 78 weeks. The period of compound administration was followed by an observation period of 28 weeks for male and high dose female rats, 29 weeks for low dose female rats and 13 weeks for mice.

There were significant positive associations between the concentrations of Michler's ketone administered and mortality in rats and mice of both sexes. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. There was distinct dose-related mean body weight depression in female rats and in mice of both sexes, and the mean body weight among dosed male rats was slightly lower than that in controls, indicating that the concentrations of Michler's ketone administered to these animals in this bioassay may have approximated the maximum tolerated concentrations.

There were significant positive associations between the concentrations of Michler's ketone administered and the incidences of hepatocellular carcinomas in both sexes of rats and in female mice and hemangiosarcomas in male mice. In all of these cases the high dose to control Fischer exact comparison was also significant.

Under the conditions of this bioassay, dietary administration of Michler's ketone was carcinogenic to male and female Fischer 344 rats and female B6C3F$_1$ mice, causing hepatocellular carcinomas, and to male B6C3F$_1$ mice, causing hemangiosarcomas.

Synonyms: bis[4-(dimethylamino)phenyl]methanone; 4,4'-bis(dimethylamino)benzophenone; p,p'-bis (dimethylamino)benzophenone; bis[p-(N,N'-dimethylamino) phenyl]ketone; tetramethyldiaminobenzophenone

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-182 Acetamide (CAS: 60-35-5)

Data considered to be inconclusive and not reportable; no Technical Report issued.

TR-183 Bioassay of Dibutyltin Diacetate for Possible Carcinogenicity (CAS No. 1067-33-0)

Dibutyltin diacetate, a widely used catalyst for polymerization reactions, was selected for bioassay by the National Cancer Institute in an effort to screen a number of organo-metallic compounds for carcinogenicity.

A bioassay for the possible carcinogenicity of dibutyltin diacetate was conducted using Fischer 344 rats and $B6C3F_1$ mice. Dibutyltin diacetate was administered in the feed, at either of two concentrations, to groups of 50 male and female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low time-weighted average dietary concentrations of dibutyltin diacetate were, respectively, 133 and 66.5 ppm for rats and 152 and 76 ppm for mice. The compound was administered for 78 weeks to rats and mice, followed by a period of no compound administration of 26 weeks for rats and 14 weeks for mice.

There were significant positive associations between the concentrations of dibutyltin diacetate administered and mortality in male rats and female mice. There were no significant positive associations between the concentrations administered and mortality in female rats or male mice. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression, relative to controls, was observed in male mice and significantly accelerated mortality, relative to controls, was observed in male rats and female mice, indicating that the concentrations of dibutyltin diacetate administered to these animals may have approximated the maximum tolerated concentrations. Since no mean body weight depression, no significantly accelerated mortality, and no other signs of toxicity were associated with administration of dibutyltin acetate to female rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

There were no neoplasms occurring in statistically significant higher incidences in dosed rats or mice when compared to their respective controls. However, there was an accidental loss of tissues from high dose female rats which precluded an evaluation of carcinogenicity in this group of animals. There was a significant positive association between the concentrations administered and the incidences of hepatocellular adenomas in females mice; however, the Fischer exact comparisons were not significant using the Bonferroni criterion. Liver neoplasms (i.e., a combination of adenomas and carcinomas) were also observed in male mice; however, the occurrence was not statistically significant.

Under the conditions of this bioassay, there was no conclusive evidence for the carcinogenicity of dibutyltin diacetate in male Fischer 344 rats or $B6C3F_1$ mice of either sex. The loss of tissues taken from high dose female rats in this bioassay precluded an evaluation of the carcinogenicity of dibutyltin diacetate to female Fischer 344 rats.

Synonyms: bis(acetyloxy)dibutylstannae; diacetoxydibutylstannae; diacetoxybutyltin; dibutyl tin diacetate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Inadequate Study
Male Mice:	Negative
Female Mice:	Negative

TR-184 Bioassay of Nitrofen for Possible Carcinogenicity (CAS No. 1836-75-5)

Nitrofen, a substituted diphenyl ether, is one of several agricultural pesticides selected for bioassay by the National Cancer Institute because of a lack of carcinogenicity data.

A bioassay for the possible carcinogenicity of nitrofen was conducted using Fischer 344 rats and $B6C3F_1$ mice. Nitrofen was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of nitrofen were 6000 and 3000 ppm for both species. The compound was administered to rats and mice for 78 weeks, followed by a period of no compound administration of 26 weeks for rats and 13 weeks for mice.

There were no significant positive associations between the concentrations of nitrofen administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Dose-related mean body weight depression, relative to controls, was observed for males and females of both species, indicating that the concentrations of nitrofen administered to the animals in this bioassay may have approximated the maximum tolerated concentrations.

None of the statistical tests for any site in rats of either sex indicated a significant positive association between compound administration and tumor incidence. There was a significant positive association between the concentration of nitrofen administered and the incidences of hepatocellular carcinomas in mice of both sexes.

In another bioassay of nitrofen for possible carcinogenicity, the compound was found to induce hepatocellular carcinomas in $B6C3F_1$ mice of both sexes and hemangiosarcomas of the liver in male $B6C3F_1$ mice. In addition, adenocarcinomas of the pancreas were induced in female Osbourne-Mendel rats.

Under the conditions of this bioassay, dietary administration of nitrofen was carcinogenic to $B6C3F_1$ mice, causing hepatocellular carcinomas in both sexes. There was no evidence for carcinogenicity in Fischer 344 rats.

Synonyms: 2,4-dichloro-1-(4-nitrophenoxy)-benzene, 2,4-dichlorophenyl-p-nitrophenyl ether, nitrophene, Tok E-25, Nip

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

Note: Nitrofen was previously studied by administration in feed to Osborne-Mendel rats and B6C3F$_1$ mice (See TR-26, reported 1979).

TR-185 Bioassay of Styrene for Possible Carcinogenicity (CAS No. 100-42-5)

Styrene, a widely used intermediate in the manufacture of plastics, elastomers, and resins, was selected for bioassay by the National Cancer Institute because of the widespread use of this compound and a lack of adequate carcinogenicity data.

A bioassay for the possible carcinogenicity of styrene was conducted using Fischer 344 rats and B6C3F$_1$ mice. Styrene was administered by gavage to groups of 50 male and 50 female animals of each species. Forty rats of each sex and twenty mice of each sex were placed on test as vehicle controls. The high, medium, and low dosages of styrene administered to rats were, respectively, 2,000, 1,000, and 500 mg/kg. The high and low dosages administered to mice were 300 and 150 mg/kg, respectively. The compound was administered for 78 weeks to high and medium dose rats, for 103 weeks to low dose rats, and for 78 weeks to mice. The period of compound administration was followed by an observation period of 27 weeks for high and medium dose rats, 1 week for low dose rats, and 13 weeks for mice.

Mortality among male and female high dose rats was significantly higher than that among their respective vehicle controls. In response to this elevated and early mortality, an additional dosed group of each sex was included in the chronic bioassay. No significant positive association was apparent between dosage and mortality among any other dosed rat groups. For mice, there was a significant positive association between mortality and the dosages of styrene administered to males, but not to females. Adequate numbers of animals in all groups, except for the high dose male and female rats, survived sufficiently long to be at risk from late-developing tumors. Slight dose-related mean body weight depression was apparent when male rats and female mice were compared to their respective vehicle controls, indicating that the dosages administered to these animals during the chronic bioassay may have approximated the maximum tolerated dosages. There was no distinct depression in mean body weight when dosed female rats and dosed male mice were compared to their respective vehicle controls. However, since there was significant accelerated mortality among high dose female rats, it is possible that the dosage administered to the medium dose female rats may have exceeded the maximum tolerated dosage.

In male mice, there was a significant positive association between styrene dosage and the incidences of a combination of adenomas and carcinomas of the lung. This finding was supported by the high dose to control Fischer exact comparison. However, the variation of the incidence of these neoplasms in historical control male mice at this laboratory does not permit a firm conclusion of carcinogenicity. There was no significant difference between tumor incidence at any other site in male mice, or at any site in rats or female mice, when dosed groups were compared to vehicle controls.

The findings of an increased incidence of a combination of adenomas and carcinomas of the lung provided suggestive evidence for the carcinogenicity of styrene in male B6C3F$_1$ mice. However, it is concluded that, under the conditions of this bioassay, no convincing evidence for the carcinogenicity of the compound was obtained in Fischer 344 rats or B6C3F$_1$ mice of either sex.

Synonyms: ethenylbenzene; vinylbenzene; vinylbenzol; styrolene; styrol; styrole; styropol; styropor; styron; cinnamene; cinnamol; phenethylene; phenylethylene; phenylethene

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Negative

TR-186 Bioassay of 4,4'-Methylenebis-(N,N-dimethyl)benzeneamine for Possible Carcinogenicity (CAS No. 101-61-1)

4,4'-Methylenebis(N,N-dimethyl)benzeneamine, a bicyclic aromatic amine and an intermediate in dye manufacture, was selected for bioassay by the National Cancer Institute because of a high incidence of bladder cancer observed among dye manufacturing industry workers.

A bioassay for the possible carcinogenicity of 4,4'-methylenebis-(N,N-dimethyl)benzeneamine was conducted using Fischer 344 rats and B6C3F$_1$ mice. 4,4'-Methylenebis-(N,N-dimethyl)benzeneamine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 4,4'-methylenebis-(N,N-dimethyl) benzeneamine were, respectively, 750 and 375 ppm for rats and 2500 and 1250 ppm for mice. The compound was administered for 59 weeks to rats and for 78 weeks to mice. The period of compound administration was followed by an observation period of 45 weeks for rats and 13 weeks for mice.

There were no significantly positive associations between the concentrations of 4,4'-methylenebis-(N,N-dimethyl)benzeneamine administered and mortality among rats or mice of either sex. Adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. There was slight dose-related mean body weight depression among female rats, the mean body weight of high dose male rats was slightly less than that for controls, and the mean body weights of dosed mice were significantly lower than their controls, indicating that the concentrations of 4,4'-methylenebis-(N,N-dimethyl)benzeneamine administered to these animals in this bioassay may have approximated the maximum tolerated concentrations.

For both male and female rats, there was a significant positive association between the concentrations of 4,4'-methylenebis-(N,N-dimethyl)benzeneamine administered and the incidences of follicular-cell carcinomas of the thyroid (i.e., 1/18, 4/50, and 21/46 in the control, low dose, and high dose males, respectively; and 0/20, 3/46, and 23/45 in the control, low dose, and high dose females, respectively). The high dose to control Fischer exact comparisons were also significant for each sex.

Liver neoplasms were observed among male and female mice. There were elevated incidences of hepatocellular adenomas in dosed mice when compared to controls (i.e., 2/20, 3/50, and 16/48 in control, low dose, and high dose males, respectively; and 1/19, 18/49, and 22/48 in control, low dose, and high dose females). The incidences of hepatocellular carcinomas in dosed mice did not differ greatly from those in controls (i.e., 3/20, 9/50, and 6/48 in control, low dose, and high dose males, respectively; and 0/19, 1/49, and 1/48 in control, low dose, and high dose females). Among both sexes of mice, there was a significant positive association between the concentrations of the chemical administered and the incidences of a combination of hepatocellular adenomas and hepatocellular carcinomas. For male mice, the Fischer exact comparisons were not significant; however, for females, both the high dose to control and the low dose to control comparisons were significant.

In both sexes of both species nonneoplastic proliferative lesions of the thyroid occurred in dosed animals but not in any of the controls.

Under the conditions of this bioassay, 4,4'-methylenebis-(N,N-dimethyl)benzeneamine was carcinogenic in Fischer 344 rats, inducing thyroid follicular-cell carcinomas in both males and females. Administration of the compound was carcinogenic in female B6C3F$_1$ mice, inducing liver neoplasms. There was no conclusive evidence that 4,4'-methylenebis-(N,N-dimethyl) benzeneamine was carcinogenic in male B6C3F$_1$ mice.

Synonyms: 4,4'-methylenebis(N,N-dimethyl)aniline; tetramethyldiaminodiphenylmethane; 4,4'-bis(dimethylamino) diphenylmethane; Tetra base; Methane base; Michler's base; Michler's hydride; Michler's methane

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Equivocal
Female Mice:	Positive

TR-187 Bioassay of 5-Chloro-o-toluidine for Possible Carcinogenicity (CAS No. 95-79-4)

5-Chloro-o-toluidine, an aromatic amine and dye intermediate, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer observed among dye manufacturing industry workers.

A bioassay for the possible carcinogenicity of 5-chloro-o-toluidine was conducted using Fischer 344 rats and B6C3F$_1$ mice. 5-Chloro-o-toluidine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 5-chloro-o-toluidine were 5,000 and 2,500 ppm for rats and 4,000 and 2,000 ppm for mice. The compound was administered in the diet for 78 weeks, followed by an observation period of 26 weeks for rats and 13 weeks for mice.

There were significant positive associations between the concentrations of 5-chloro-o-toluidine administered and mortality among male and female mice, but not among rats of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Distinct mean body weight depression was apparent when dosed female rats and dosed mice of both sexes were compared to their controls, indicating that the concentrations administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no mean body weight depression, relative to controls, no significantly accelerated mortality, and no signs of toxicity other than fatty metamorphosis of the liver were associated with administration of 5-chloro-o-toluidine to male rats, it is possible that these animals may have been able to tolerate a higher dietary concentration of the compound.

There was a significant positive association between the concentration of 5-chloro-o-toluidine administered to male rats and the incidence of adrenal pheochromocytomas in these animals; however, neither of the Fischer comparisons was significant. None of the other statistical tests for tumors at any site in male and female rats indicated a significant positive association between dosage and incidence.

In mice of both sexes there were significant positive associations between concentration administered and the incidence of hemangiosarcomas. In addition, the high dose to control Fischer exact comparisons for both sexes were significant. The Cochran-Armitage tests were also significant and positive for the incidences of hepatocellu-

lar carcinomas in both sexes of mice. For males and females the high dose to control Fischer exact comparisons were significant, and for females the low dose to control comparison was also significant.

Under the conditions of this bioassay, 5-chloro-o-toluidine was carcinogenic to B6C3F$_1$ mice, inducing hemangiosarcomas and hepatocellular carcinomas in both males and females. There was no conclusive evidence of the carcinogenicity of the compound in Fischer 344 rats.

Synonyms: 5-chloro-2-methylbenzeneamine; p-chloro-o-aminotoluene; 4-chloro-2-aminotoluene; 2-amino-4-chlorotoluene; o-amino-p-chlorotoluene; 5-chloro-2-methylaniline; 2-methyl-5-chloroaniline; 1-amino-2-methyl-5-chlorobenzene; 1-amino-3-chloro-6-methylbenzene; Fast Red KB Base; Azogene Fast Red KB; Brentamine Fast Red KB Base; Naphthosol Fast Red Base; Naphthanil Red KB Base

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-188 Sodium Iodomethanesulfonate (CAS: 126-31-8)

Data considered to be inconclusive and not reportable; no Technical Report issued.

TR-189 Bioassay of p-Chloroaniline for Possible Carcinogenicity (CAS No. 106-47-8)

p-Chloroaniline, a dye and chemical intermediate, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer observed among dye manufacturing industry workers.

A bioassay for the possible carcinogenicity of p-chloroaniline was conducted using Fischer 344 rats and B6C3F$_1$ mice. p-Chloroaniline was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of p-chloroaniline were, respectively, 500 and 250 ppm for rats and 5000 and 2500 ppm for mice. The compound was administered in the diet for 78 weeks, followed by an observation period of 24 weeks for rats and 13 weeks for mice.

There were no significant positive associations between the dietary concentrations of p-chloroaniline administered and mortality in female rats or in mice of either sex; however, there was a significantly positive association between concentration and mortality in male rats. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression, in relation to controls, was observed in high dose female rats and dosed mice of both sexes, indicating that the concentration of p-chloroaniline administered to these animals may have approximated the maximum tolerated concentrations. Although splenic lesions were observed in male rats, no mean body weight depression relative to controls was associated with administration of p-chloroaniline to these animals. Therefore, it is possible that these animals may have been able to tolerate a higher dietary concentration of the compound.

The only neoplastic lesions found that might be related to administration of the compound were mesenchymal tumors in the spleens of male rats and hemangiomatous tumors in mice. In male rats, there was a significant positive association between compound administration and the incidences of fibroma or fibrosarcoma of the spleen. The incidences of these tumors were not significantly elevated when compared to those in control rats, but the rarity of these tumors in male Fischer 344 rats (0/360 in historical male control rats in this laboratory) strongly suggests a chemically related effect. In addition, three sarcomas of other types were found in high dose male rats. In mice of both sexes, hemangiomas and hemangiosarcomas were found at elevated incidences, when compared to control mice, in the spleen, liver, kidney, and multiple body sites. The increased incidences in dosed mice were statistically related to dose but were not statistically significant when compared directly to matched control animals. In comparison to historical control data, the incidences of hemangiomatous tumors in the dosed mice were elevated, but not greatly. The evidence was considered insufficient to conclusively relate the hemangiomatous tumors in mice to compound administration. Nonneoplastic proliferative and chronic inflammatory lesions were also found in the spleens of dosed rats and mice.

The findings of small numbers of fibromas and sarcomas in the spleens of male rats was considered strongly suggestive of carcinogenicity because of the rarity of these tumors in the spleens of control rats. Hemangiomatous tumors in dosed mice may also have been associated with administration of p-chloroaniline. However, it is concluded that, under the conditions of this bioassay, sufficient evidence was not found to establish the carcinogenicity of p-chloroaniline for Fischer 344 rats or B6C3F$_1$ mice.

Synonyms: 4-chlorobenzeneamine; 4-chlorophenylamine; 4-chloroaniline; 4-CA; 1-amino-4-chlorobenzene

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Equivocal

TR-190 Bioassay of p-Nitrosodiphenylamine for Possible Carcinogenicity (CAS No. 156-10-5)

p-Nitrosodiphenylamine is used to accelerate the vulcanization of rubber. It is also used as an intermediate in the manufacture of dyes and pharmaceutical compounds and as an inhibitor of polymerization during the production of vinyl monomers such as styrene.

A bioassay for the possible carcinogenicity of p-nitrosodiphenylamine was conducted using Fischer 344 rats and $B6C3F_1$ mice. p-Nitrosodiphenylamine was administered in the feed, at either of two concentrations, to groups of 50 male and female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of p-nitrosodiphenylamine were, respectively, 5000 and 2500 ppm for rats. The high and low time-weighted average concentrations for mice were 9000 and 4254 ppm, respectively. The compound was administered for 78 weeks to rats, for 50 weeks to high dose mice and for 57 weeks to low dose mice. The period of compound administration was followed by an observation period of 27 weeks for rats and 35 weeks for mice.

There were significant positive associations between the concentrations of p-nitrosodiphenylamine administered and mortality among male and female mice, but not for rats of either sex. Although 19/50 high dose male mice and 21/50 high dose female mice died before week 52, adequate numbers of mice and rats survived sufficiently long to be at risk from late-developing tumors. The toxicity observed in mice and the dose-related mean body weight depression apparent in male and female rats indicated that the concentrations of p-nitrosodiphenylamine administered to these animals in this bioassay may have approached or exceeded the maximum tolerated concentrations.

In male rats, there was a significant positive association between concentration administered and the incidence of a combination of hepatocellular carcinomas and neoplastic nodules. In addition, both the high dose to control and the low dose to control Fischer exact comparisons were significant. There was also a significant positive association between concentration administered and the incidence of alveolar/bronchiolar adenomas in male rats; however, neither of the Fischer exact comparisons were significant. There were no positive, significant statistical tests for tumor incidence at any site in female rats.

Due to the large number of early deaths among high dose mice of both sexes, the statistical conclusion concerning carcinogenicity was based on comparisons between the low dose and control groups. The incidence of hepatocellular carcinomas was significantly higher among the low dose males than among their controls. Although hepatocellular neoplasms were observed in dosed females, there were no tumors occurring with a statistically increased incidence when low dose females were compared to their controls.

Under the conditions of this bioassay, p-nitrosodiphenylamine was carcinogenic when administered in the diet to male $B6C3F_1$ mice, causing hepatocellular carcinomas. The chemical was also carcinogenic in male Fischer 344 rats, causing liver neoplasms. No evidence was provided for the carcinogenicity of p-nitrosodiphenylamine in female $B6C3F_1$ mice or in female Fischer 344 rats.

Synonyms: 4-nitroso-N-phenylbenzeneamine; 4-nitrosodiphenylamine; p-nitroso-N-phenylaniline; TKB

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Negative

TR-191 Bioassay of Technical Grade Bis(2-chloro-1-methylethyl) ether for Possible Carcinogenicity (CAS No. 108-60-1)

Bis(2-chloro-1-methylethyl) ether is a beta-haloether and a byproduct of propylene oxide and propylene glycol manufacture.

A bioassay of technical grade bis(2-chloro-1-methylethyl) ether for possible carcinogenicity was conducted by administering the test chemical by gavage to F344 rats.

Groups of 50 rats of each sex were administered a solution of bis(2-chloro-1-methylethyl) ether in corn oil 5 days per week at either 100 or 200 mg/kg/day for 103 weeks. Vehicle controls consisted of groups of 50 rats of each sex that were administered the corn oil alone. Untreated-control groups of the same size were also used. All surviving rats were killed at week 104 or 105.

Mean body weights of the dosed groups of male and female rats were lower than those of the corresponding vehicle-control groups throughout most of the study and were dose related. Similarly, survivals of the high-dose males and of both the high- and low-dose females were lower than those of the corresponding vehicle controls and were dose related. Almost all animals in the high-dose groups died by the end of the bioassay.

No tumors occurred in the dosed groups of rats of either sex at incidences that were significantly higher than those of the vehicle-control groups.

It is concluded that under the conditions of this bioassay, the technical-grade test material, bis(2-chloro-1-methylethyl) ether, was not carcinogenic for F344 rats of either sex.

Synonym: bis(2-chloro-isopropyl) ether

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative

Note: Bis(2-chloro-1-methylethyl)ether was subsequently tested in B6C3F$_1$ mice by gavage (See TR-239, reported 1982).

TR-192 Bioassay of Malathion for Possible Carcinogenicity (CAS No. 121-75-5)

Malathion is an organophosphate insecticide considered to be suitable as a substitute for certain uses of DDT. U.S. consumption in 1974 was 16 million pounds, surpassing that of all other organophosphate insecticides except methyl parathion. Household applications accounted for approximately 10% of that volume.

A bioassay of malathion for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats.

Groups of 49 or 50 rats of each sex were fed diets containing 2,000 or 4,000 ppm malathion for 103 weeks and were then observed for an additional 2 or 3 weeks. Matched controls consisted of 50 untreated rats of each sex. All surviving rats were killed at 105 or 106 weeks.

No tumors occurred in the dosed groups of rats of either sex at incidences that could be related clearly to administration of the test chemical. Compound-related toxic effects were not observed in female rats at the doses used, but in males decreased mean body weights, increased mortality, gastritis, and gastric ulcers were dose related.

It was concluded that under the conditions of this bioassay, malathion was not carcinogenic in male or female rats, but the females may not have received a maximum tolerated dose.

Synonym: S-(1,2-bis(ethoxycarbonyl)-ethyl) *O,O*-dimethylphosphorodithioate

Report Date: 1979

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative

Note: Malathion was previously tested in Osborne-Mendel rats and B6C3F$_1$ mice administered in feed (See TR-24, reported 1978).

TR-193 Bioassay of Resperine for Possible Carcinogenicity (CAS No. 50-55-5)

A bioassay for possible carcinogenicity of resperine, an antihypertensive drug for human use, was conducted by administering the test chemical in feed to F344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered resperine at two doses, 5 ppm and 10 ppm, for 103 weeks and then observed for an additional 2 weeks. Matched controls consisted of groups of 50 untreated rats and 50 untreated mice of each sex. All surviving animals were killed and necropsied at the end of 104 or 105 weeks.

The significant effects that could be related to administration of resperine at the doses used were decreased body weight and increased tumor formation in dosed male rats and in mice of both sexes. Dosed male rats had an increased incidence of adrenal medullary pheochromocytomas. Among dosed mice, some males developed undifferentiated carcinomas of the seminal vesicles, which rarely occur in control mice, and females had an increased incidence of mammary cancer.

It was concluded that, under the conditions of the bioassay, resperine was carcinogenic in male rats and in mice of both sexes, producing three different kinds of cancers. Resperine was not carcinogenic for female rats, but they may not have received a high enough dose for maximum test sensitivity.

Report Date: November 1982

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-194 Bioassay of Selenium Sulfide (Gavage) for Possible Carcinogenicity (CAS No. 7446-34-6)

Selenium sulfide is an ingredient in dandruff shampoos used in concentrations of 1% in products sold over-the-counter and 2.5% in products which are available by prescription only. Prescription shampoos have been shown in clinical studies to be of therapeutic value against dandruff. An antimitotic mechanism of action is suggested by data showing that selenium sulfide decreases the rate of incorporation of radioactively labeled thymidine into the DNA of dermal epithelial cells. Approximately 200 kg of selenium sulfide is estimated to be used annually for this purpose.

A bioassay of selenium sulfide for possible carcinogenicity was conducted by administering this substance by gavage to F344 rats and B6C3F$_1$ mice.

Groups of 50 rats and 50 mice of each sex were administered selenium sulfide suspended in 0.5% aqueous carboxymethylcellulose 7 days per week for 103 weeks at either 3 or 5 mg/kg/day for rats and 20 or 100 mg/kg/day for mice. As vehicle controls, groups of 50 rats and 50 mice of each sex were administered only the 0.5% aqueous carboxymethylcellulose. Similar groups of untreated controls also were used. All surviving rats and mice were killed and necropsied at week 104 or 105.

The significant effects that could be related to administration of selenium sulfide at the doses used were

decreased body weight and increased tumor formation in female mice and in rats of each sex. Dosed rats and female mice had an increased incidence of hepatocellular carcinomas and adenomas. Dosed female mice also had an increased incidence of alveolar/bronchiolar carcinomas and adenomas.

Under the conditions of this bioassay, selenium sulfide was carcinogenic for F344 rats and female $B6C3F_1$ mice, including hepatocellular carcinomas in male and female rats and female mice and alveolar/bronchiolar carcinomas and adenomas in female mice. Selenium sulfide was not carcinogenic for male mice; but they have been able to tolerate higher doses.

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Negative
Female Mice:	Positive

Note: Selenium sulfide was subsequently tested in ICR Swiss mice administered dermally (See TR-197, reported 1980).

TR-195 Bioassay of Fluometuron for Possible Carcinogenicity (CAS No. 2164-17-2)

Fluometuron is a phenylurea herbicide used in agriculture to control broad-leaved and grass weeds in cotton and sugarcane fields. The area of heaviest use is the Mississippi delta. Applications of low concentrations selectively kill weeds.

A bioassay of the phenylurea herbicide fluometuron for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were fed diets containing 125 or 250 ppm of fluometuron for 103 weeks, and groups of 50 mice of each sex were fed diets containing 500 or 1,000 ppm of fluometuron for 103 weeks. Matched controls consisted of groups of 50 untreated rats and 25 untreated mice of each sex. All surviving animals were killed at 103 to 105 weeks.

Splenomegaly observed in rats in the subchronic studies influenced selection of the doses for the chronic study; however, no splenic effects were observed in the chronic study.

Mean body weights of the dosed groups of male and female rats and mice were essentially the same as those of the corresponding control groups. Survival of dosed groups of rats and mice was similar to that of the corresponding control groups. Similarities between mean body weights and survival between dosed and control animals in the chronic study suggest that these animals could have tolerated higher doses.

The only possible carcinogenic effects from compound administration were in male mice. Incidences of hepatocellular carcinomas or adenomas in male mice were dose related, and the incidence in the high-dose group was marginally higher than that in the corresponding matched controls or pooled controls from concurrent studies.

Under the conditions of this bioassay, fluometuron was not carcinogenic for F344 rats or for female $B6C3F_1$ mice. Equivocal results were obtained for male $B6C3F_1$ mice which may have had an increased incidence of hepatocellular tumors. Because of the equivocal findings and because both rats and mice may have been able to tolerate higher doses, it is concluded that additional testing of fluometuron for carcinogenicity is warranted.

Synonym: 1,1-dimethyl-3-(a,a,a-trifluoro-m-tolyl) urea

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Equivocal
Female Mice:	Negative

TR-196 Bioassay of Cinnamyl Anthranilate for Possible Carcinogenicity (CAS No. 87-29-6)

A bioassay of cinnamyl anthranilate (a synthetic flavoring agent) for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats and 50 mice of each sex were fed the test chemical in diets containing 15,000 or 30,000 ppm for 103 weeks and then observed for an additional 2 or 3 weeks. Controls consisted of groups of 50 untreated rats and 50 untreated mice of each sex. All surviving animals were killed and necropsied at 105 to 107 weeks.

Mean body weights of the dosed male and female rats and mice were lower than those of the corresponding controls throughout the bioassay, and weight decrements were dose related. Mortality in rats and mice of either sex was not affected by administration of the test chemical.

In male rats, adenocarcinomas or adenomas of the renal cortex and acinar-cell carcinomas or adenomas of the pancreas were found in low incidences in dosed rats but not in control rats. In direct comparisons with matched control groups, the incidences of these tumors were not significantly increased; however, because these tumors rarely occur spontaneously in aging F344 rats, they were considered to be related to compound administration. Similar pancreatic or renal tumors have not been detected among 634 historical-control male F344 rats at the same laboratory.

In the female rats, no tumors occurred at incidences that could be clearly related to the administration of the test chemical.

In both male and female mice, the incidences of hepatocellular carcinomas or adenomas were dose

related ($P<0.001$) and significant ($P \leq 0.001$) in direct comparisons of dosed and control groups.

It was concluded that under the conditions of this bioassay cinnamyl anthranilate was carcinogenic for male and female B6C3F$_1$ mice, inducing increased incidences of hepatocellular carcinomas or adenomas. The test chemical was also carcinogenic for male F344 rats, inducing low incidences of acinar-cell carcinomas or adenomas of the pancreas and adenocarcinomas or adenomas of the renal cortex. Cinnamyl anthranilate was not carcinogenic for female F344 rats.

Synonyms: 2-aminobenzoic acid, 3-phenyl-2-propenyl ester; anthranilic acid, cinnamyl ester

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Negative
Male Mice:	Positive
Female Mice:	Positive

TR-197 Bioassay of Selenium Sulfide (Dermal Study) for Possible Carcinogenicity (CAS No. 7446-34-6)

Selenium is an essential nutrient, and various selenium compounds have industrial and medical uses.

The possible carcinogenicity of selenium sulfide (a component in shampoos) was investigated by applying a suspension of this substance to the skin of ICR Swiss mice. Groups of 50 mice of each sex were treated by applying 0.5 mg or 1.0 mg selenium sulfide three times a week for 86 weeks to a clipped 2- x 3-cm dorsal surface. The selenium sulfide was suspended in 0.05 ml saline solution containing 0.5% carboxymethylcellulose.

Mean body weights of all dosed and control groups were comparable throughout the study. Amyloidosis, previously reported as a cause of death in Swiss mice, was a factor in the deaths of most treated and control mice after 1 year, and the study was terminated after 88 weeks when the majority of animals in all dosed and control groups had died.

Under the conditions of this bioassay, dermal application of selenium sulfide did not produce a carcinogenic effect in ICR Swiss mice, but the study was limited by the relatively short lifespan of this strain of mouse.

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Mice:	Negative
Female Mice:	Negative

Note: Selenium Sulfide was previously tested in F344 and B6C3F$_1$ mice administered by gavage (See TR-194, reported 1980).

TR-198 Bioassay of a Mixture of 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin and 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin (Gavage) for Possible Carcinogenicity (CAS No. 57653-85-7, CAS No. 19408-74-3)

Hexachlorodibenzo-p-dioxins (HCDD) are formed during the manufacture of certain chlorophenols. They have been found in trichlorophenol, tetrachlorophenol, and pentachlorophenol and in the chlorophenol-derived herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). From 1967 to 1970, the concentration of HCDD in commercial pentachlorophenol ranged from 0.03 to 38 ppm. Since then, HCDD levels in pentachlorophenol have been less than 1 ppm.

A bioassay of a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (HCDD) for possible carcinogenicity was conducted by administering the test material by gavage to Osborne-Mendel rats and B6C3F$_1$ mice for 104 weeks.

Fifty rats and 50 mice of each sex were administered HCDD suspended in a vehicle of 9:1 corn oil-acetate 2 days per week for 104 weeks at doses of 1.25, 2.5, or 5 μg/kg/wk for rats and male mice and 2.5, 5, or 10 μg/kg/wk for female mice. Seventy-five rats and 75 mice of each sex served as vehicle controls. In addition, one untreated control group containing 25 rats and 25 mice of each sex was present in the HCDD treatment room, and one untreated control group containing 25 rats and 25 mice of each sex was present in the vehicle control room. All surviving animals were killed at 105 to 108 weeks.

In rats, a dose-related depression in mean body weight gain became evident in the males after week 68 of the bioassay and in the females after week 33. In mice, the mean body weight gain in the dosed groups was comparable with that of the vehicle control groups. No other toxic clinical signs were reported in either the rats or the mice. Administration of HCDD had no adverse effect on the survival of either species.

In male rats, hepatocellular carcinomas or neoplastic nodules occurred at low incidences that were dose related ($P = 0.003$). In a direct comparison, the incidence of these tumors in the high-dose group was higher ($P = 0.022$) than that in the corresponding vehicle-control groups, but the Bonferroni requirement of $P = 0.017$ for the multiple comparison of three dosed groups with a control group was not met.

In female rats, hepatocellular carcinomas, adenomas, or neoplastic nodules occurred at incidences that were dose related ($P<0.001$), and in direct comparisons the incidences of these tumors in the mid- and high-dosed groups were significantly higher ($P = 0.006$ and $P<0.001$, respectively) than those in the corresponding vehicle-control group.

In male mice, hepatocellular carcinomas or adenomas occurred at incidences that were dose related ($P = 0.001$), and in a direct comparison the incidence of these tumors in the high-dose group was significantly higher ($P = 0.001$) than that in the corresponding vehicle-control group.

In female mice, hepatocellular carcinomas or adenomas occurred at incidences that were dose-related (P = 0.002), and the incidence of these tumors in the high-dose group was significantly higher (P = 0.004) than that in the corresponding vehicle-control group.

Complex nonneoplastic toxic liver lesions were seen in all dosed groups of rats and mice. Compound-associated hyperplastic lesions of the lung were also found in both male and female rats.

Under the conditions of this bioassay, HCDD administered by gavage was carcinogenic, causing increased incidences of hepatocellular carcinomas or neoplastic nodules in female Osborne-Mendel rats and inducing hepatocellular carcinomas and adenomas in male and female $B6C3F_1$ mice. HCDD was not demonstrated to be carcinogenic for male rats.

Synonym: HCDD

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Rats:	Equivocal
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

Note: 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin and 1,2,3,7,8.9-Hexachlorodibenzo-p-dioxin were subsequently tested in Swiss-Webster mice administered dermally (See TR-202, reported 1980).

TR-199 Bioassay of Selsun® for Possible Carcinogenicity

A bioassay of Selsun® for possible carcinogenicity was conducted by applying this substance dermally to ICR Swiss mice. Selsun®, an antidandruff shampoo, contains 2.5% selenium sulfide.

Groups of 50 mice of each sex were exposed to 0.05 ml of 25% or 50% Selsun® in distilled water three times a week on a 2- x 3-cm clipped dorsal surface. Vehicle controls consisted of 50 mice of each sex that were clipped and treated with distilled water. Untreated controls consisted of 50 mice of each sex that were only clipped. Surviving mice were killed and necropsied at week 88.

Mean body weights of untreated control, vehicle control, low-dose, and high-dose groups were comparable throughout the bioassay. Amyloidosis was a factor in the deaths of most animals after 1 year. In male mice, alveolar/bronchiolar carcinomas or adenomas occurred with a dose-related trend that was significant (P = 0.008). The result of the Fisher exact test comparing the incidence in the high-dose group with that in the vehicle controls is also significant, but the incidence of the high-dose group, when compared with that of the untreated controls, is not significant.

Under the conditions of this bioassay, dermal application of Selsun® was not carcinogenic for ICR Swiss mice. The study was limited, however, by the relatively short lifespan of this strain of mouse.

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Mice:	Negative
Female Mice:	Negative

TR-200 Bioassay of 2,6-Toluenediamine Dihydrochloride for Possible Carcinogenicity (CAS No. 15481-70-6)

2,6-Toluenediamine is used as an intermediate in the production of dyes for furs and textiles, and of flexible polyurethane foams and elastomers. A bioassay of 2,6-toluenediamine dihydrochloride for possible carcinogenicity was conducted by feeding diets containing the test chemical to F344 rats and $B6C3F_1$ mice.

Groups of 50 rats of each sex were fed the test chemical at two doses, 250 or 500 ppm, for 103 weeks and observed for 1 additional week. Groups of 50 mice of each sex were fed the test chemical at two doses, 50 or 100 ppm, for 103 weeks and then observed for 1 additional week. Groups of 50 untreated rats and 50 untreated mice of each sex were used as matched controls. All surviving animals were killed and necropsied at 104 weeks.

Weight gain depression was less than 10% for dosed groups of male rats and male and female mice, when compared with controls. Mean body weight gain was depressed 17% in low-dose female rats and 27% in high-dose female rats. Mortality was not increased in rats or mice of either sex by the test chemical. No clinical evidence indicated that mice of either sex received a maximum tolerated dose of the compound.

In male rats, islet-cell adenomas of the pancreas and neoplastic nodules or carcinomas of the liver occurred in dose-related trends that were significant using the Cochran-Armitage test (P = 0.025 and P = 0.037, respectively). The results of the Fisher exact test were not significant for either lesion. The occurrences of tumors in dosed female rats were not significantly different from those in control rats.

Significant results in the negative direction were observed in the incidences of C-cell tumors of the thyroid in male rats and of fibroadenomas of the mammary gland in female rats.

In male mice, in the low-dose group, lymphomas occurred at an incidence significantly higher (P = 0.046) than that of the corresponding control group; however, the incidence was not significant when the Bonferroni criterion for multiple comparison was used.

The occurrence of hepatocellular carcinomas in female mice was dose related, but the result of the Fisher exact test comparing the incidence in the high-dose group with that in the controls was not significant.

It was concluded that, under the conditions of the bioassay, 2,6-toluenediamine dihydrochloride was not

carcinogenic for male and female F344 rats or for male and female $B6C3F_1$ mice.

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-201 Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (CAS No. 1746-01-6) in Swiss-Webster Mice (Dermal Study)

2,3,7,8-Tetrachlorodibenzo-p-dioxin occurs as a highly toxic impurity found in herbicides containing 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4,5-T derivatives, as well as in other chemicals synthesized using 2,4,5-trichlorophenol.

The herbicide 2,4,5-T has been marketed in the United States since 1948. Production increased sharply between 1960 and 1970 when a 1:1 mixture of 2,4,5-T and 2,4-dichlorophenoxyacetic acid (2,4-D) was used as a defoliant in Vietnam under the names of "herbicide agent orange, herbicide orange, agent orange, and orange". During this 10-year period, about 106 million pounds of 2,4,5-T were sprayed.

A carcinogenesis bioassay was conducted by applying an acetone suspension of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the clipped backs of 30 male and female Swiss-Webster mice 3 days per week for 99 or 104 weeks. Similar groups were pretreated with 1 application of 50 μg dimethylbenzanthracene (DMBA) in 0.1 ml acetone 1 week before TCDD administration began. Female mice received 0.005 μg TCDD per application, and the male mice received 0.001 μg TCDD. As vehicle controls, 45 mice of each sex received 0.1 ml acetone three times per week. Thirty animals of each sex were used as untreated controls.

Throughout the bioassay, mean body weights of the male and female mice administered TCDD, or TCDD following DMBA, were essentially the same as those of the corresponding vehicle control group. Mean body weights of dosed and vehicle control groups of males were less than those of the untreated control group throughout the study; for the females, mean body weights were less than the untreated controls during the first 80 weeks.

In female mice, the incidences of fibrosarcoma in the integumentary system in dosed groups with TCDD were significantly ($P = 0.007$) higher than that in the corresponding controls (2/41, 5%; 8/27, 30%). An increase in the same tumor type, although not statistically significant ($P = 0.084$), was also observed in the male mice (3/42, 7%; 6/28, 21%).

In the DMBA-TCDD experiment, failure to have included groups skin painted with only DMBA precluded interpretation of these results.

Under the conditions of this bioassay, 2,3,7,8-tetrachlorodibenzo-p-dioxin applied to the skin was not carcinogenic for male Swiss-Webster mice (the increase of fibrosarcomas in the integumentary system may have been associated with the skin application of TCDD). TCDD was carcinogenic for female Swiss-Webster mice causing fibrosarcomas in the integumentary system.

Synonyms: 2,3,7,8-TCDD; TCDD

Report Date: February 1982

Note: 2,3,7,8-Tetrachlorodibenzo-p-dioxin was subsequently tested in Osborne-Mendel rats and $B6C3F_1$ mice by gavage (See TR-209 reported 1982).

TR-202 Bioassay of a Mixture of 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin and 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin (Dermal Study) for Possible Carcinogenicity (CAS No. 57653-85-7; CAS No. 19408-74-3)

Hexachlorodibenzo-p-dioxin (HCDD) is formed as a byproduct during the manufacture of certain chlorophenols and has been found in trichlorophenol, tetrachlorophenol, pentachlorophenol and in the chlorophenol-derived herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). From 1967 to 1970, the concentration of HCDD in commercial pentachlorophenol ranged from 0.03 to 38 ppm. Since then, HCDD levels in pentachlorophenol have been reduced to less than 1 ppm.

A bioassay of a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins (HCDD) for possible carcinogenicity was conducted by dermal application of a suspension of this substance to Swiss-Webster mice.

HCDD (0.01 μg) suspended in 0.1 ml acetone was applied to the backs of 30 mice of each sex 3 days per week for 104 weeks. During the first 16 weeks, doses were 0.005 μg HCDD per application. An additional 30 mice of each sex were pretreated with one application of 50 μg DMBA in 0.1 ml acetone 1 week before the initiation of the HCDD applications. As vehicle controls, 45 mice of each sex received 0.1 ml of acetone three times per week. Thirty animals of each sex served as untreated controls. Mean body weights of all test and vehicle control mice were comparable throughout the bioassay; mean body weights of untreated controls were higher than those of the test and vehicle-control groups.

In male mice, the incidence of alveolar/bronchiolar carcinomas in the group administered only HCDD was significantly higher ($P = 0.045$) than that in the vehicle-control group; however, the incidence was not significantly higher when compared with untreated controls.

In female mice, the incidences of fibrosarcomas of the skin were significantly higher (P = 0.044) in animals administered HCDD (both with and without pretreatment with DBMA) than in the untreated-control group; however, when the incidences were compared with those of the vehicle controls (relative risk = 3.037) the results were not significant.

Under the conditions of this bioassay, HCDD was not carcinogenic for male or female Swiss-Webster mice.

Synonym: HCDD

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Mice:	Negative
Female Mice:	Negative

Note: Hexachlorodibenzo-p-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin were previously tested in Swiss-Webster mice administered dermally (See TR-198, reported 1980).

TR-203 Bioassay of Phenol for Possible Carcinogenicity (CAS No. 108-95-2)

Phenol ranked 38th in production among U.S. chemicals in 1978 with annual production of 2.38 billion pounds. Approximately 90% of the phenol produced is used in the manufacture of phenolic (phenol formaldehyde) resins, caprolactam, bisphenol A, alkyl phenol, and adipic acid. The remainder of the phenol is used to produce an assortment of end products, including salicylic acid, phenacetin, dyes, metal cleaners, disinfectants, antiseptics, photographic chemicals, wood preservatives (pentachlorophenol), paints, paint and varnish removers, and agricultural chemicals (2,4-D and parathion).

A bioassay of phenol to test for possible carcinogenicity was conducted by providing this substance in drinking water to F344 rats and $B6C3F_1$ mice. Groups of 50 rats and 50 mice of each sex were given drinking water containing 2,500 or 5,000 ppm phenol for 103 weeks. As matched controls, groups of 50 rats and 50 mice of each sex received tap water.

A dose-related depression in mean body weight gain occurred in rats and mice of each sex. Rats and mice given water containing phenol drank less than did the corresponding controls. A dose-related decrease in water consumption was observed for mice.

An increased incidence of leukemia or lymphomas was detected in male rats and may have been associated with the administration of phenol. Although the incidence of these tumors in the low-dose group was significantly higher than that in controls, the incidence in the high-dose group was not. Thus an association with administration of phenol was not established.

Under the conditions of this bioassay, phenol was not carcinogenic for either male or female F344 rats or male and female $B6C3F_1$ mice.

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-204 Bioassay of Benzoin for Possible Carcinogenicity (CAS No. 119-53-9)

A bioassay of benzoin for possible carcinogenicity was conducted by incorporating the test chemical in diets of F344 rats and $B6C3F_1$ mice. Benzoin is used as a photopolymerization catalyst, chemical intermediate, and flavor ingredient.

Groups of 50 male rats were fed diets containing 125 or 250 ppm benzoin for 104 weeks, and similar groups of female rats received feed containing 250 or 500 ppm. Groups of 50 mice of each sex were fed diets containing 2,500 or 5,000 ppm, benzoin for 104 weeks. Groups of 50 untreated rats and mice of each sex were used as matched controls. Rats and mice of either sex probably could have tolerated higher doses. An increased incidence of lymphomas or leukemia occurred in dosed male rats, but the observed dose-related trend was not statistically significant.

Mean body weights and clinical signs of low-dose, high-dose, and control male and female rats and male mice were comparable throughout the study, After week 44, mean body weights of dosed female mice were slightly lower (10% or less) than those of the controls.

The incidences of lymphomas that occurred in male mice varied with each dose but were not statistically significant when compared with those of matched controls.

Lymphomas or leukemias occurred in low-dose female mice at an incidence that was significant when compared with the matched controls. However, because the incidence of lymphomas or leukemias in the high-dose female mice was not significant, the occurrence of these tumors was not clearly related to administration of the test compounds.

Under the conditions of this bioassay, benzoin was not carcinogenic for F344 rats or $B6C3F_1$ mice.

Synonym: 2-hydroxy-1,2-diphenylethanone

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Rats:	Negative
Female Rats:	Negative
Male Mice:	Negative
Female Mice:	Negative

TR-205 Bioassay of 4,4'-Oxydianiline for Possible Carcinogenicity (CAS No. 101-80-4)

4,4'-Oxydianiline is used in the manufacture of high temperature resistant metal adhesives, molding and machine parts, and insulators. A bioassay of this chemical for possible carcinogenicity was conducted by feeding diets containing 200, 400, or 500 ppm of the test chemical to groups of 50 male or female F344 rats and 150, 300, or 800 ppm to groups of 50 male or female B6C3F$_1$ mice for 104 weeks. Matched controls consisted of 50 untreated rats and 50 untreated mice of each sex. All surviving animals were killed at 104 to 105 weeks.

A dose-related decrement in mean body weight gain was observed for all groups of dosed rats and mice. Survival was significantly shortened in the high-dose female rats and in the low- and mid-dose female mice.

In male and female rats, hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose-related, and the incidences in all dosed groups (except low-dose females) were higher than those in the controls. The occurrence of follicular-cell adenomas or carcinomas of the thyroid was dose-related. Among groups of male and female rats, the incidences in the mid- and high-dose groups of either sex were significantly higher than those of the corresponding controls.

In male and female mice, adenomas in the harderian glands occurred in all dosed groups at incidences that were significantly higher than the incidence in the matched controls.

In low-dose male mice and in high-dose female mice, hepatocellular adenomas or carcinomas occurred at incidences significantly higher than those in the matched controls.

In female mice, follicular-cell adenomas in the thyroid occurred with a positive linear trend, and in a direct comparison the incidence in the high-dose group was also significantly higher than that in the controls.

Tumors occurring among male mice at increased incidences which could not be statistically related to the chemical were adenomas in the pituitary and hemangiomas of the circulatory system.

Under the conditions of this bioassay, 4,4'-oxydianiline was carcinogenic for male and female F344 rats, inducing hepatocellular carcinomas or neoplastic nodules and follicular-cell adenomas or carcinomas of the thyroid. 4,4'-Oxydianiline was also carcinogenic for male and female B6C3F$_1$ mice, including adenomas in the harderian glands, hepatocellular adenomas or carcinomas in both sexes, and follicular-cell adenomas in the thyroid of females.

Report Date: August 1980

Levels of Evidence of Carcinogenicity:

Male Rats:	Positive
Female Rats:	Positive
Male Mice:	Positive
Female Mice:	Positive

TR-206 Carcinogenesis Bioassay of 1,2-Dibromo-3-chloropropane (CAS No. 96-12-8) in F344 Rats and B6C3F$_1$ Mice (Inhalation Study)

1,2-Dibromo-3-chloropropane (DBCP), a contaminant (0.05%) of the flame retardant tris(2,3-dibromopropyl)-phosphate, has been used primarily as a soil fumigant to control nematodes. Unlike other halogenated nematocides, DBCP can be applied to soil without damaging growing perennials. Since it is slightly soluble in water at the concentrations used (30 ppm), DBCP can be either injected directly into the soil or added to irrigation water. By 1972, an estimated 12.3 million pounds were being used annually; in 1977, a total of 832,000 pounds were used in California, mostly on grapes and tomatoes.

A carcinogenesis bioassay of technical grade 1,2-dibromo-3-chloropropane (DBCP), which contained trace amounts of epichlorohydrin and 1,2-dibromoethane, was conducted by exposing groups of 50 F344 rats and B6C3F$_1$ mice of each sex by inhalation to concentrations of 0.6 or 3.0 ppm DBCP for 6 hours per day, 5 days per week, for 76 to 103 weeks. Untreated chamber controls consisted of 50 rats and 50 mice of each sex. Surviving high-dose rats were killed at week 84. Surviving high-dose female mice and low- and high-dose male mice were killed at week 76. Low-dose rats and female mice were killed at week 104.

Accelerated mortality occurred in the high-dose groups of both species. Early deaths of high-dose rats and mice were associated with respiratory tract tumors. Interference with breathing and metastasis to the brain were major contributing factors in these deaths. Among male mice, accelerated mortality occurred in low-dose and control groups as well as in the high-dose group. Urogenital infection appeared to be associated with these deaths.

Carcinomas, squamous-cell carcinomas, and adenocarcinomas of the nasal cavity and squamous-cell papillomas of the tongue each occurred in high-dose male rats at incidences significantly higher than those in the corresponding controls. Adenocarcinomas, adenomas, adenomatous polyps, and squamous-cell papillomas of the nasal cavity and adenomatous polyps of the nasal turbinates occurred in low-dose male rats with significantly increased incidences relative to controls.

Carcinomas and adenocarcinomas of the nasal cavity, squamous-cell papillomas of the tongue, squamous-cell papillomas and carcinomas (combined) of the pharynx, and adenomas of the adrenal cortex each occurred in high-dose female rats at incidences significantly higher than those in the corresponding controls. Also, adenomas and squamous-cell papillomas of the nasal cavity, adenomas of the adrenal cortex, and fibroadenomas of the mammary gland were increased significantly in low-dose female rats when compared with controls.

Adenocarcinomas of the nasal cavity in high-dose female mice, papillary carcinomas in low-dose female

mice, and carcinomas, squamous cell carcinomas of the nasal cavity, and alveolar/bronchiolar adenomas or carcinomas of the lung in high-dose male and female mice occurred at incidences significantly higher than those in the corresponding controls.

Exposure to DBCP vapor was also associated with toxic tubular nephropathy in rats and mice of either sex and with proliferative changes in the nasal mucosa, lung, and forestomach in mice.

Under the conditions of this bioassay, DBCP was carcinogenic for male and female F344/N rats, including increased incidences of nasal cavity tumors and tumors of the tongue in both sexes, and cortical adenomas in the adrenal glands of females. DBCP was carcinogenic in male and female B6C3F$_1$ mice, including increased incidences of nasal cavity tumors and lung tumors.

Synonyms: DBCP; dibromochloropropane; Nemagon; Fumazone

Report Date: March 1982

Note: 1,2-Dibromo-3-chloropropane was previously tested in Osborne-Mendel rats and B6C3F$_1$ mice by gavage (See TR-28, reported 1978).

TR-207 Carcinogenesis Bioassay of Cytembena (CAS No. 21739-91-3)

A carcinogenesis bioassay of cytembena, a cytostatic agent, was conducted by injecting intraperitoneally 7 or 14 mg/kg into groups of 50 male and 50 female F344 rats and 12 or 24 mg/kg into groups of 50 male or 50 female B6C3F$_1$ mice three times per week for 104 weeks. Groups of 50 rats and 50 mice of both sexes served as vehicle controls.

Mean body weights of dosed and vehicle-control rats were comparable throughout the bioassay. Mean body weights of dosed and vehicle-control mice were comparable for the first 73 weeks of the bioassay; mean body weight of the high dose male mice was slightly lower than that of the vehicle controls after 73 weeks, and that of the high-dose female mice was lower after week 87.

In dosed male rats, mesotheliomas in the tunica vaginalis and malignant mesotheliomas in multiple organs occurred with dose-related trends and at incidences in each of the dosed groups which were significantly higher than those in the vehicle control rats.

In dosed female rats, fibroadenomas in the mammary gland occurred with a dose-related trend and at a significantly higher incidence in the high-dose group than in vehicle control rats.

Under the conditions of this bioassay, cytembena was carcinogenic for male and female F344 rats, causing increased incidences of mesotheliomas in the tunica vaginalis and in multiple organs of males and fibroadenomas in the mammary gland of females. Cytembena was not carcinogenic for male or female B6C3F$_1$ mice.

Synonyms: cytembene; 2-butenoic acid; 3-bromo-4-(4-methoxyphenyl)-4-oxo-, sodium salt

Report Date: May 1981

TR-208 Carcinogenesis Bioassay of FD & C Yellow No. 6 (CAS No. 2783-94-0)

A carcinogenesis bioassay was conducted using groups of 50 male and 50 female F344 rats and B6C3F$_1$ mice which were fed diets containing 12,500 or 25,000 ppm FD & C Yellow No. 6, a widely used food colorant, for 103 weeks. Groups of 90 male and 90 female rats and 50 male and 50 female mice served as undosed controls.

Throughout the study, mean body weights of high-dose female rats and all low-dose groups were comparable with those of the controls, but mean body weights of high-dose male rats and high-dose male and female mice were slightly lower (10% or less) than those of the controls.

No compound-related neoplastic or nonneoplastic lesions were observed in the rats.

The incidence of hepatocellular carcinomas in low-dose male mice was significantly higher than that in the controls, but the lack of a significant increase in high-dose males and the variability of liver tumors in B6C3F$_1$ male mice precluded clearly relating the occurrence of these tumors to the administration of FD & C Yellow No. 6.

Under the conditions of this bioassay, there was no clear evidence of the carcinogenicity of FD & C Yellow No. 6 in F344 rats or B6C3F$_1$ mice of either sex.

Synonym: Sunset Yellow FCF

Report Date: May 1981

TR-209 Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (CAS No. 1746-01-6) in Osborne-Mendel Rats and B6C3F$_1$ Mice (Gavage Study)

A carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a contaminant in several phenoxy herbicides, was conducted by administering TCDD by gavage to Osborne-Mendel rats and B6C3F$_1$ mice for 104 weeks.

Fifty rats and mice of each sex were given TCDD suspended in a vehicle of 9:1 corn oil-acetone 2 days per week for 104 weeks at doses of 0.01, 0.05, or 0.5 μg/kg/wk for rats and male mice and 0.04, 0.2, or 2.0 μg/kg/wk for female mice. Seventy-five rats and 75 mice of each sex served as vehicle controls. One untreated control group containing 25 rats and 25 mice of each sex was present in the TCDD treatment room, and one untreated control group containing 25 rats and 25 mice of each sex was present in the vehicle-control room. All surviving animals were killed at 105 to 108 weeks.

In rats, a dose-related depression in mean body weight gain was observed in the males after week 55 of the bioassay and in the females after week 45. In mice, the mean body weight gain in the dosed groups was comparable to that of the vehicle-control groups.

In male rats, increased incidences of follicular-cell adenomas in the thyroid were dose related and were significantly (P = 0.001) higher in the high-dose group than in the vehicle controls (1/69, 1%; 5/48, 10%; 6/50, 12%; 10/50, 20%). Similarly in the female rats, an increase (though not statistically significant) was seen in the high-dose group (3/73, 4%; 2/45, 4%; 1/49, 2%; 6/47, 13%).

In female rats, the incidence of neoplastic nodules of the liver in the high-dose group was significantly (P = 0.006) higher than that in the vehicle control group (5/75, 7%; 1/49, 2%; 3/50, 6%; 12/49, 24%).

In male and female mice, incidences of hepatocellular carcinomas were dose related and the incidences in the high-dose groups were significantly (P = 0.002 and 0.014, respectively) higher than those in the corresponding vehicle control groups (males: 8/73, 11%; 9/49, 18%; 9/49, 16%; 17/50, 34%; females: 1/73, 1%; 2/50, 4%; 2/48, 4%; 6/47, 13%).

In female mice, follicular-cell adenomas in the thyroid occurred at dose-related incidences, and were significantly (P = 0.009) higher in the high-dose groups than those in the vehicle controls (0/69, 0%; 3/50, 6%; 1/47, 2%; 5/46, 11%).

Increased incidences of toxic hepatitis related to the administration of the test chemical were detected among high-dose rats and high-dose mice of each sex.

Under the conditions of this bioassay, 2,3,7,8-tetrachlorodibenzo-p-dioxin was carcinogenic for Osborne-Mendel rats, including follicular-cell thyroid adenomas in males and neoplastic nodules of the liver in females. TCDD was also carcinogenic for $B6C3F_1$ mice, including hepatocellular carcinomas in male and females and follicular-cell thyroid adenomas in females.

Synonyms: 2,3,7,8-TCDD; TCDD

Report Date: February 1982

Note: 2,3,7,8-Tetrachlorodibenzo-p-dioxin was previously tested in Swiss-Webster mice administered dermally (See TR-201, reported 1982).

TR-210 Carcinogenesis Bioassay of 1,2-Dibromoethane (CAS No. 106-93-4) in F344 Rats and $B6C3F_1$ Mice (Inhalation Study)

A carcinogenesis bioassay of 1,2-dibromoethane, a widely used nematocide and leaded gasoline additive, was conducted by exposing groups of 50 F344 rats and $B6C3F_1$ mice of each sex by inhalation to concentrations of 10 or 40 ppm of the 1,2-dibromoethane for 78-103 weeks. Untreated controls consisted of 50 rats and 50 mice of each sex exposed in chambers to ambient air.

Throughout the study, mean body weights of high-dose rats and high-dose mice of either sex were lower than those of the corresponding untreated controls. Survival of the high-dose rats of either sex and of the low- and high-dose female mice was significantly shorter than that in the corresponding controls.

The principal cause of early death in control and dosed male mice was ascending, suppurative urinary tract infection that resulted in necrotic, ulcerative lesions around the urethral opening, chronic or suppurative cystitis (often with urinary tract obstruction), and ascending suppurative pyelonephritis.

Carcinomas and adenocarcinomas of the nasal cavity were observed with significantly increased incidences (P<0.001) in high-dose rats of either sex relative to controls. The incidences of adenocarcinomas and adenomas of the nasal cavity were also significantly increased (P<0.001) in low-dose rats of either sex. Adenomatous polyps of the nasal cavity showed significantly increased incidence (P<0.001) in low-dose male rats. The combined incidence of alveolar/bronchiolar adenomas and carcinomas was statistically significant (P = 0.024) for high-dose female rats.

Hemangiosarcomas of the circulatory system (mainly spleen) and mesotheliomas of the tunica vaginalis occurred in high-dose male rats with significantly increased incidences (P<0.001) relative to controls.

The incidence of fibroadenomas of the mammary gland was significantly elevated (P<0.001) in dosed female rats relative to controls.

The incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma were significantly increased (P<0.001) in high-dose male mice relative to controls. These tumors were also increased in high-dose female mice (P = 0.007 for adenomas and P<0.001 for carcinomas).

Hemangiosarcomas occurred in low- and high dose female mice at incidences significantly greater (P<0.001) than the incidence in the controls (0/50). High-dose female mice also had significantly increased incidences of subcutaneous fibrosarcomas (P<0.001) and of nasal cavity carcinomas (P = 0.013). Low-dose female mice also showed a significantly increased incidence (P<0.001) of mammary gland adenocarcinomas.

Exposure to 1,2-dibromoethane was also associated with hepatic necrosis and toxic nephropathy in rats of either sex, testicular degeneration in male rats, retinal degeneration in female rats, and epithelial hyperplasia of the respiratory system in mice.

Under the conditions of this bioassay, 1,2-dibromoethane was carcinogenic for F344 rats, causing increased incidences of carcinomas, adenocarcinomas, adenomas of the nasal cavity, and hemangiosarcomas of the circulatory system in males and females; mesotheliomas of the tunica vaginalis and adenomatous polyps of the nasal cavity in males; and fibroadenomas of the mammary gland and alveolar/bronchiolar adenomas and carcinomas (combined) in females. 1,2-Dibromoethane was carcinogenic for $B6C3F_1$ mice, causing alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas in males and females; and hemangiosarcomas of the circulatory system, fibrosarcomas in the sub-

cutaneous tissue, carcinomas of the nasal cavity, and adenocarcinomas of the mammary gland in females.

Synonyms: ethylene dibromide; EDB; ethylene bromide

Report Date: March 1982

Note: 1,2-Dibromoethane was previously tested in Osborne-Mendel rats and B6C3F$_1$ mice by gavage (See TR-86, reported 1978).

TR-211 Carcinogenesis Studies of C.I. Acid Orange 10 (CAS No. 1936-15-8) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

Carcinogenesis studies of 80% pure C.I. Acid Orange 10 (a monoazo textile dye) were conducted by feeding to groups of 50 male and 50 female F344/N rats diets containing 1,000 or 3,000 ppm C.I. Acid Orange 10 for 103 weeks. Groups of 50 male and 50 female B6C3F$_1$ mice were fed diets containing 3,000 or 6,000 ppm for 103 weeks. Groups of 90 male and 90 female untreated rats and 50 male and 50 female untreated mice served as controls.

Mean body weights and clinical signs of control and dosed rats and mice were comparable. Because no toxic effects or consistent weight differences were observed, the rats and mice may have been able to tolerate higher doses.

In male rats with neoplastic nodules of the liver, the dose response trend was positive ($P<0.05$) and the incidence in the 3,000 ppm group was increased ($P<0.05$) compared to controls (control, 5/90, 6%; low dose, 3/50, 6%; high dose, 8/50, 16%). One male rat in the high dose group had both a neoplastic nodule and a carcinoma of the liver. This marginal increase in liver cell neoplasms may have been associated with the dietary administration of C.I. Acid Orange 10.

For both dose groups of male and female rats, leukemia was significantly ($P<0.05$) decreased in a dose related ($P<0.005$) trend (male: 22/90, 24%; 4/50, 8%; 3/50, 6%; female: 16/88, 18%; 2/50, 4%; 0/50).

No compound-related nonneoplastic or neoplastic lesions were observed in the female rats or in mice of either sex.

For 103 weeks C.I. Acid Orange 10 was given in the diets of male and female F344/N rats (0, 0.1, or 0.3%) and of male and female B6C3F$_1$ mice (0, 0.3%, or 0.6%). Under these conditions, there was no evidence of carcinogenicity for male and female F344/N rats or for male and female B6C3F$_1$ mice.

Synonym: 7-hydroxy-8-(phenylazo)-1,3-naphthalenedisulfonic acid, disodium salt

Report Date: October 1987

TR-212 Carcinogenesis Bioassay of Di(2-ethylhexyl)adipate (CAS No. 103-23-1) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

Di(2-ethylhexyl)adipate is a plasticizer used to give flexibility to vinyl plastics. A carcinogenesis bioassay was conducted by feeding diets containing 12,000 or 25,000 ppm of di(2-ethylhexyl)adipate to groups of 50 male and 50 female F344 rats and 50 male and 50 female B6C3F$_1$ mice for 103 weeks. Groups of 50 undosed rats and mice of each sex served as controls. All surviving animals were killed at 104 to 107 weeks.

Mean body weights of high-dose rats and mice of either sex were lower than those of the controls throughout the study.

Compound administration was not associated with tumor formation in F344 rats of either sex.

Hepatocellular carcinomas or adenomas occurred in mice of both sexes in a dose-related fashion at incidences that were significantly higher for high-dose males and for low- and high-dose females than those in the controls. When compared with the incidence in historical laboratory control mice, however, the liver tumors in male mice could not be clearly related to compound administration.

Under the conditions of this bioassay, di(2-ethylhexyl)adipate was not carcinogenic for F344 rats. Di(2-ethylhexyl)adipate was carcinogenic for female B6C3F$_1$ mice, causing increased incidences of hepatocellular carcinomas, and was probably carcinogenic for male B6C3F$_1$ mice, causing hepatocellular adenomas.

Synonyms: bis(2-ethylhexyl)adipate; DEHA; octyl adipate; diocytl adipate; DOA

Report Date: March 1982

TR-213 Carcinogenesis Bioassay of Butyl Benzyl Phthalate (CAS No. 85-68-7) in F344/N Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of butyl benzyl phthalate, a plasticizer for vinyl chloride plastics, was accomplished by feeding diets containing 6,000 or 12,000 ppm of the phthalate to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex for 28 to 103 weeks.

Mean body weights of dosed female rats and mice of each sex were lower than those of the control animals throughout most of the study.

After week 14, an increasing number of dosed male rats died as a result of an unexplained internal hemorrhaging, and all surviving male rats were killed at week 29 to 30. Because of compound-related mortality, butyl benzyl phthalate was not adequately tested for carcinogenicity in male F344/N rats.

Mononuclear cell leukemias occurred at a statistically significant ($P=0.011$) increased incidence in the high-

dose group of female rats when compared with the control group and with a significantly (P = 0.006) increasing trend (controls 7/49, 14%; low-dose 7/49, 14%; high-dose 18/50, 36%). The incidence in the high-dose group and the overall trend remained statistically significant (P = 0.008 and P = 0.019) when compared with the historical incidence for F344/N female rats with leukemia at this laboratory (77/ 399, 19%). Further, this leukoproliferation was generally characterized by splenomegaly and often by hepatomegaly.

Administration of butyl benzyl phthalate was not associated with increased incidences of any type of tumor among male or female mice.

Tumor rates were decreased in female rats for fibroadenomas of the mammary glands (20/49, 14/49, 9/50) and in male mice for lymphomas of the hematopoietic system (13/50, 11/49, 4/50) and for alveolar/bronchiolar adenomas or carcinomas (17/50, 11/49, 8/50).

Under the conditions of this bioassay, butyl benzyl phthalate was probably carcinogenic for female F344/N rats, causing an increased incidence of mononuclear cell leukemias. The male F344/N rat study was considered inadequate for evaluation due to compound-related toxicity and early mortality. Butyl benzyl phthalate was not carcinogenic for $B6C3F_1$ mice of either sex.

Synonyms: BBP; benzyl butyl phthalate; phthalic acid; benzyl butyl ester; Santicizer 160

Report Date: August 1982

TR-214 Carcinogenesis Bioassay of Caprolactam (CAS No. 105-60-2) in F344 Rats and $B6C3F_1$ Mice (Feed Study)

A carcinogenesis bioassay of caprolactam, a chemical intermediate used in the production of nylon 6, was conducted by feeding diets containing 3,750 or 7,500 ppm caprolactam to groups of 50 male or female F344 rats and 7,500 or 15,000 ppm to groups of 50 male or female $B6C3F_1$ mice for 103 weeks. Control groups consisted of 50 undosed rats and 50 undosed mice of each sex.

Throughout the bioassay, mean body weight gains for dosed rats and mice of either sex were decreased when compared with those of the controls. No other compound-related effects were observed.

Under the conditions of this bioassay, caprolactam was not carcinogenic for F344 rats or $B6C3F_1$ mice.

Synonyms: aminocaproic lactam; 2-oxohexamethylenimine

Report Date: March 1982

TR-215 Carcinogenesis Bioassay of Bisphenol A (CAS No. 80-05-7) in F344 Rats and $B6C3F_1$ Mice (Feed Study)

A carcinogenesis bioassay of bisphenol A, an intermediate used in the manufacture of epoxy, polycarbonate, and polyester-styrene resins, was conducted by feeding diets containing 1,000 or 2,000 ppm of the test chemical to groups of 50 F344 rats of either sex, 1,000 or 5,000 ppm to groups of 50 male $B6C3F_1$ mice, and 5,000 or 10,000 ppm to groups of 50 female $B6C3F_1$ mice for 103 weeks. Groups of 50 rats and 50 mice of either sex served as controls.

Mean body weights of rats of either sex and of high- and low-dose female mice and high-dose male mice were lower than those of the controls throughout the study. Since food consumption of dosed female rats was only 70% to 80% that of the controls throughout most of this study, reduced body weight gain was probably due to reduced food consumption. Food consumption by dosed male rats was 90% that of controls. Food consumption among all groups of mice appear to be similar.

Leukemias occurred at increased incidences in dosed rats of both sexes. In male rats, the dose-related (13/50, 12/50, 23/50) trend was statistically significant (P = 0.021) by a Cochran-Armitage test, but neither the trend nor the increase in the high-dose group was significant by life table analyses, which adjust for survival differences among groups. The increased incidences in dosed female rats were also not statistically significant (7/50, 13/50, 12/50).

Interstitial-cell tumors of the testes occurred at statistically significant incidences in low- and high-dose male rats; however, since this lesion normally occurs at a high incidence in aging F344 male rats, the increased incidence observed in this study was not considered compound related (35/49, 48/50, 46/49).

In male mice, there was an increased incidence of leukemias or lymphomas (2/49, 9/50, 5/50), but this increase was not statistically significant.

A compound-related increased incidence of multinucleated giant hepatocytes was observed in male mice (1/49, 41/49, 41/50), but there was no increase of liver tumors in male mice.

The marginally significant increase in leukemias in male rats, along with an increase (not statistically significant) in leukemias in female rats and a marginally significant increase in the combined incidence of lymphomas and leukemias in male mice, suggests that exposure to bisphenol A may be associated with increased cancers of the hematopoietic system. A statistically significant increase in interstitial-cell tumors of the testes in male rats was also suggestive of carcinogenesis, but was not considered to be convincing evidence of a compound-related effect because this lesion normally occurs at a high incidence in aging F344 rats.

Under the conditions of this bioassay, there was no convincing evidence that bisphenol A was carcinogenic for F344 rats or $B6C3F_1$ mice of either sex.

Synonym: 4,4'-isopropylidenediphenol

Report Date: March 1982

TR-216 Carcinogenesis Bioassay of 11-Aminoundecanoic Acid (CAS No. 2432-99-7) in F344 Rats and B6C3F$_1$ Mice

11-Aminoundecanoic acid is the monomer used in the manufacture of the polyamide, nylon-11. Aminoundecanoic acid is synthesized through a series of reactions from ricinoleic acid isolated from castor bean oil.

Nylon-11 is used in automobile parts, industrial fabrics (e.g. filter bags, work clothes, and netting), and brushes because of its resistance to vibration and shock and its stability when in contact with fuels. Nylon-11 resins are approved by the U.S. Food and Drug Administration for use on food contact films.

A carcinogenesis bioassay of 11-aminoundecanoic acid was carried out by administering diets containing 7,500 or 15,000 ppm of 11-aminoundecanoic acid to F344 rats and B6C3F$_1$ mice. Groups of 50 rats and 50 mice of either sex were administered the test chemical for 104 weeks (rats) or 103 weeks (mice). Controls consisted of 50 untreated rats and 50 untreated mice of each sex.

Nonneoplastic effects included dose-related decreases in mean body weight gain and survival for male rats and for mice of each sex; a dose-related increased incidence of hyperplasia of the transitional epithelium of the kidney and urinary bladder in rats of each sex; and mineralization of the kidney in dosed mice of each sex.

Neoplastic nodules of the liver in dosed male rats (control 1/50, 2%; low dose 9/50, 18%; high dose 8/50, 16%; $P<0.01$) and transitional-cell carcinomas of the urinary bladder in high-dose male rats (control 0/48, 0%; low dose 0/48, 0%; high dose 7/49, 14%: $P<0.01$) were observed at significantly increased incidences compared with controls. Malignant lymphomas occurred at a significantly ($P<0.05$) increased rate in low-dose male mice (control 2/50, 4%; low dose 9/50, 18%; high dose 4/50, 8%).

Under the conditions of this bioassay, 11-aminoundecanoic acid was carcinogenic for male F344 rats, inducing neoplastic nodules in the liver and transitional-cell carcinomas in the urinary bladder. The test chemical was not carcinogenic for female F344 rats. No clear evidence was found for the carcinogenicity of 11-aminoundecanoic acid in B6C3F$_1$ mice of either sex, although the increase in malignant lymphoma in male mice may have been associated with administration of 11-aminoundecanoic acid.

Report Date: May 1982

TR-217 Carcinogenesis Bioassay of Di(2-ethylhexyl)phthalate (CAS No. 117-81-7) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

A bioassay of di(2-ethylhexyl)phthalate, the most commonly used plasticizer for polyvinylchloride polymers, for possible carcinogenicity was conducted by feeding diets containing 6,000 or 12,000 ppm of the test chemical to groups of 50 male and 50 female F344 rats and 3,000 or 6,000 ppm to groups of 50 male and 50 female B6C3F$_1$ mice for 103 weeks. Controls consisted of 50 untreated rats and 50 untreated mice of either sex.

Mean body weights of dosed male rats (high- and low-dose), high-dose female rats, and dosed female mice (high- and low-dose) were marginally-to-moderately lower than those of the corresponding controls at the end of the chronic study, reflecting a decrease in body weight gain. Food consumption was reduced slightly in rats of either sex, whereas there was no apparent difference among the mouse groups.

Female rats and male and female mice administered di(2-ethylhexyl)phthalate had significantly higher incidences of hepatocellular carcinomas than those observed in the controls (rats – males: 1/50, 2%; 1/49, 2%; 5/49, 10%; females – 0/50, 0%; 2/49, 4%; 8/50, 16%, $P=0.003$; mice – males: 9/50, 18%; 14/48, 29%; 19/50, 38%, $P=0.022$; females: 0/50, 0%; 7/50, 14%; $P=0.006$, 17/50, 34%, $P<0.001$). Further, a statistically significant positive trend for hepatocellular carcinomas occurred in female rats ($P=0.002$) and in male ($P=0.018$) and female ($P<0.001$) mice.

In addition, di(2-ethylhexyl)phthalate caused a statistically significant increased incidence of male rats with either hepatocellular carcinomas or neoplastic nodules (3/50, 6%; 6/49, 12%; 12/49, 24%; $P=0.010$).

Degeneration of the seminiferous tubules was observed in the high-dose male rats (1/49, 2%; 2/44, 5%; 43/48, 90%) and in the high-dose male mice (1/49, 2%; 2/48 4%; 7/49, 14%). Hypertrophy of cells in the anterior pituitary was also found at increased incidences in the high-dose male rats (1/46, 2%; 0/43, 0%; 22/49, 45%).

Under the conditions of this bioassay, di(2-ethylhexyl)phthalate was carcinogenic for F344 rats and B6C3F$_1$ mice, causing increased incidences of female rats and male and female mice with hepatocellular carcinomas, and inducing an increased incidence of male rats with either hepatocellular carcinomas or neoplastic nodules.

Synonym: DEHP

Report Date: March 1982

TR-218 Monitoring Guidelines for the Conduct of Carcinogen Bioassays

Note to the Reader: This document was prepared as a result of a discussion group on quality assurance conducted at the National Cancer Institute in 1979.

Report Date: 1981

TR-219 Carcinogenesis Bioassay of 2,6-Dichloro-p-Phenylenediamine (CAS No. 609-20-1) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of 2,6-dichloro-p-phenylenediamine, a chemical intermediate, was conducted in groups of 50 F344 rats and B6C3F$_1$ mice of either sex. Male rats were fed diets containing 1,000 or 2,000 ppm 2,6-dichloro-p-phenylenediamine and female rats were fed 2,000 or 6,000 ppm for 103 weeks. Mice were fed 1,000 or 3,000 ppm of the test chemical for 103 weeks and observed for an additional 8 weeks. Controls consisted of 50 untreated rats and 50 untreated mice of each sex.

Throughout the study, mean body weights of dosed rats and mice of either sex were lower than those of the corresponding controls. A dose-related weight gain depression was particularly pronounced for rats.

Ectopic hepatocytes were observed at an increased incidence in the pancreas and nephrosis was observed in increased severity in dosed rats of either sex when compared with the corresponding controls. No increase in any tumor type was observed in treated male or female rats when compared to controls.

Increased incidences of liver tumors were observed in mice of both sexes. In male mice, the incidence of hepatocellular adenomas exhibited a significant positive dose-related trend ($P = 0.002$), and the increased incidence of hepatocellular adenomas was statistically significant in the high-dose group (4/50, 7/50, 15/50: $P = 0.005$). The combined incidence of hepatocellular adenomas and carcinomas showed a significant positive dose-related trend ($P = 0.004$) and was statistically significant in the high-dose group (16/50, 19/50, 29/50: $P = 0.008$).

In female mice, hepatocellular carcinomas exhibited a significant positive dose-related trend ($P = 0.025$), but no single dose group had a statistically significant increased incidence of either adenomas (4/50, 4/50, 9/50; high-dose effect: $P = 0.12$) or carcinomas (2/50, 2/50, 7/50; high-dose effect: $P = 0.08$) alone. When the incidences of hepatocellular adenomas and carcinomas were combined (6/50, 6/50, 16/50), these data gave a positive dose-related trend ($P = 0.004$) and were statistically significant in the high-dose group ($P = 0.014$).

Under the conditions of this bioassay, 2,6-dichloro-p-phenylenediamine was carcinogenic for male and female B6C3F$_1$ mice, causing increased incidences of combined hepatocellular adenomas and carcinomas, and for male B6C3F$_1$ mice, causing an increased incidence of hepatocellular adenomas alone. 2,6-Dichloro-p-phenylenediamine was not carcinogenic for male or female F344 rats.

Report Date: March 1982

TR-220 Carcinogenesis Bioassay of C.I. Acid Red 14 (CAS No. 3567-69-9) in F344/N Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of textile grade C.I. Acid Red 14 (67%-71% purity) was conducted by feeding diets containing 6,000 or 12,500 ppm of this dye for 103-104 weeks to groups of 50 male F344 rats, 12,500 or 25,000 ppm to groups of 50 female F344 rats, and 3,000 or 6,000 ppm to groups of 50 B6C3F$_1$ mice of either sex. Groups of 90 untreated rats of either sex and 50 untreated mice of either sex served as controls.

Throughout the study, mean body weights of dosed rats of either sex and dosed female mice were comparable with those of the controls, while the mean body weight of high-dose male mice was slightly lower than that of the controls.

Fourteen male rats in the low-dose group and 2 in the high-dose group accidentally drowned between weeks 84 and 103; 56% and 60% of these groups survived to terminal kill compared with 78% of the controls. These losses may have reduced the sensitivity of the assay in male rats.

Rats and mice may have tolerated higher doses, but the slight depression of mean body weight in high-dose male mice and the non-neoplastic lesions observed in dosed female mice and in rats of both sexes suggest that doses administered in this study could be considered maximum tolerated doses.

Endometrial stromal polyps of the uterus were observed in high-dose female rats at an incidence significantly higher ($P = 0.008$) than that seen in the controls (controls: 9/87, 10%; low-dose: 11/50, 22%; high-dose: 14/50, 28%). However, the observed incidence of this tumor in the dosed groups was similar to the historical rate in untreated female F344 rats at this laboratory (65/286, 23%; range 10%-37%). Hence, the increased incidence of this lesion is not regarded as being associated with the administration of C.I. Acid Red 14.

Administration of C.I. Acid Red 14 to mice was not associated with an increased incidence of any tumor type.

Under the conditions of this bioassay, C.I. Acid Red 14 was not carcinogenic for F344 rats or B6C3F$_1$ mice of either sex.

Synonym: 4-hydroxy-3-(4-Sulfo-1-naphthalenyl)azo-1-naphthalenesulfonic acid, disodium

Report Date: March 1982

TR-221 Carcinogenesis Bioassay of Locust Bean Gum (CAS No. 9000-40-2) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of locust bean gum, a widely used food stabilizer, was conducted by feeding diets containing 25,000 or 50,000 ppm of the test substance to

50 F344 rats and 50 B6C3F$_1$ mice of either sex for 103 weeks. Groups of 50 untreated rats and mice of either sex served as controls.

Mean body weights of high- and low-dose rats of either sex, of low-dose male mice, and of high- and low-dose female mice were comparable with those of the controls; mean body weights of high-dose male mice were slightly lower than those of controls. No other compound-related clinical signs or effects on survival were observed. Although the rats and mice might have been able to tolerate higher doses, 50,000 ppm (5%) is the recommended maximum concentration of a test chemical mixed in feed according to the guidelines of the Bioassay Program.

Although alveolar/bronchiolar adenomas occurred in low-dose male mice at a significantly ($P = 0.017$) higher incidence than that in the controls (7/50, 17/50, 11/50), no significant statistical results were obtained when the combined incidence of animals with either alveolar/bronchiolar adenomas or carcinomas was analyzed (14/50, 21/50, 14/50). Cortical adenomas in the adrenal gland of female rats occurred with a statistically significant ($P = 0.042$) positive trend (1/50, 4/50, 6/50), but comparisons between test groups and the control group were not statistically different.

Under the conditions of this bioassay, locust bean gum was not carcinogenic for male or female F344 rats or B6C3F$_1$ mice.

Report Date: February 1982

TR-222 Carcinogenesis Bioassay of Disperse Yellow 3 (CAS No. 2832-40-8) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of C.I. Disperse Yellow 3 (87.6% dye), a textile dye, was conducted by feeding diets containing 5,000 or 10,000 ppm of the test substance to groups of 50 F344 rats of either sex for 103 weeks. Similar groups of 50 B6C3F$_1$ mice received diets containing 2,500 or 5,000 ppm of the test substance for 103 weeks. Groups of 50 untreated rats and mice of each sex served as controls.

Throughout the bioassay, mean body weights of dosed rats and mice of either sex were lower than those of the controls. Survival of dosed rats of either sex was significantly greater than that of the corresponding controls. No other compound-related clinical signs or effects on survival were observed.

A significant increase in neoplastic nodules of the liver occurred in dosed male rats as compared to controls (controls 1/49, 2%; low-dose 15/50, 30%; $P<0.001$; high-dose, 10/50, 20%; $P<0.01$). No increase was observed for female rats.

Stomach tumors, rare in F344 rats (10/2960, 0.3%), were found in the dosed male rats: one adenocarcinoma and a sarcoma in a high-dose male and in the low-dose group a squamous cell papilloma, fibrosarcoma, adenoma, and mucinous adenocarcinoma. The incidence of these tumors was not significantly greater than that in controls; thus, the association between the administration of C.I. Disperse Yellow 3 and the stomach tumors in male rats is not clearly established.

Negative trends in the incidences of certain primary tumors in dosed rats included: decreased lymphocytic leukemia in both sexes; decreased malignant mesothelioma and C-cell carcinoma of the thyroid in males; and decreased pituitary chromophobe adenoma and endometrial stromal polyps in females.

Hepatocellular adenomas occurred in dosed female mice at incidences significantly higher than that in the controls (control 0/50, 0%; low-dose 6/50, 12%, $P<0.05$; high-dose 12/50, 24%, $P<0.001$). The incidences of hepatocellular carcinomas were also higher in the dosed female mice than in the controls, but the increased incidences were not statistically significant (2/50, 4/50, 5/50). A significantly ($P<0.05$) lower incidence of hepatocellular adenomas was detected among low-dose (7/50, 1/49, 7/49) male mice.

Alveolar/bronchiolar adenomas occurred in high-dose male mice at an incidence significantly ($P<0.05$) higher than that in the controls (control 2/50, 4%; low-dose 6/49, 12%; high-dose 9/49, 18%). However, the high-dose effect was not significant when adenomas and carcinomas were combined; the incidence among low-dose female mice was significantly reduced as compared with controls. Thus, the incidence of alveolar/bronchiolar adenomas among males is not considered to be related to treatment with C.I. Disperse Yellow 3.

Malignant lymphomas occurred in a dose-related ($P<0.05$) trend in female mice and at incidences greater ($P<0.05$) in the high-dose group than that in the controls (10/50; 16/50; 19/50). However, because of the range of variability in the historical incidence of this tumor and because of the lack of a similar effect in male mice or in male and female rats, this increase was not regarded as being unequivocally related to the administration of C.I. Disperse Yellow 3.

Under the conditions of this bioassay, C.I. Disperse Yellow 3 was considered to be carcinogenic for male F344 rats, causing an increased incidence of neoplastic nodules of the liver; this dye was not carcinogenic for female F344 rats. In addition, the stomach tumors found in the male rats may have been induced by the administration of the test chemical. C.I. Disperse Yellow 3 was carcinogenic for female B6C3F$_1$ mice, as evidenced by the increased incidence of hepatocellular adenomas; C.I. Disperse Yellow 3 was not carcinogenic for male B6C3F$_1$ mice. Also, the increased incidence of malignant lymphoma in female mice may have been associated with the administration of C.I. Disperse Yellow 3.

Synonyms: Disperse Fast Yellow 6; Acetamine Yellow CG

Report Date: May 1982

TR-223 Carcinogenesis Studies of Eugenol (CAS No. 97-53-0) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Carcinogenesis studies of eugenol (>99% pure), a widely used flavor additive and chemical intermediate, were conducted by feeding diets containing 6,000 or 12,500 ppm of eugenol to groups of 50 female F344/N rats and by feeding diets containing 3,000 or 6,000 ppm to groups of 50 male F344/N rats and B6C3F$_1$ mice of each sex for 103 weeks. Groups of 40 rats and 50 mice of each sex served as controls. Dose levels selected for the two year studies were based on thirteen-week (91-day) studies in which dietary concentrations for the six groups ranged from 0 to 12,500 ppm. Other than a -10% difference from controls in body weights in the 12,500 ppm male rats, no chemically related gross or histopathologic effects were observed.

In the two-year studies, with the exception of the high dose female rats and female mice, final body weights of the treated groups were comparable to their respective controls. No significant differences in survival were apparent for any of the eight groups receiving eugenol and for the appropriate controls. Food consumption among groups was not different in comparison with controls— rats: males ≥97%, females ≥91%; mice: males ≥94%,females≥90%.

There were no significant observable differences between treated and control groups of rats for either nonneoplastic (toxic) lesions or neoplasms that could be attributed to eugenol. Increases in tumor incidences were diagnosed for low dose male rats with alveolar, bronchiolar adenomas or carcinomas (combined), for C-cell adenomas of the thyroid gland in low dose female rats, and for endometrial stromal polyps of the uterus in high dose female rats. Fibroadenomas of the mammary gland were decreased in dosed groups of female rats compared with controls. None of these differences were considered to be associated with the dietary administration of eugenol.

In male mice, the low dose animals had an increased incidence ($P<0.05$) of both hepatocellular adenomas (control, 4/50; low dose, 13/50; high dose, 10/49) and hepatocellular carcinomas (10/50, 20/50, 9/49) when compared with control animals. A significant increase in hepatic neoplasms was not observed in high dose animals. No single liver tumor type was observed in female mice with a statistically significant increased incidence. When the incidences of female mice with hepatocellular adenoma or carcinoma were combined (2/50, 7/49, 9/49), there was a dose-related positive trend and the incidence of liver neoplasms in high dose animals was higher than in controls ($P<0.05$).

Eugenol was given in the diets of female F344/N rats (0, 0.6, or 1.25%) and of male F344/N rats and male and female B6C3F$_1$ mice (0, 0.3, or 0.6%) for 103 weeks. Under these experimental conditions, there was *no evidence of carcinogenicity* observed for male or female rats. For mice there was *equivocal evidence of carcinogenicity* since eugenol caused increased incidences of both carcinomas and adenomas of the liver in male mice at the 3,000 ppm dietary level and because eugenol was associated with an increase in the combined incidences of hepatocellular carcinomas or adenomas in female mice.

Synonym: 1-allyl-3-methoxy-4-hydroxybenzene

Report Date: December 1983

TR-224 Carcinogenesis Bioassay of Tara Gum (CAS No. 39300-88-4) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of tara gum, a potential stabilizer for cosmetics and foods, was conducted by feeding diets containing 25,000 or 50,000 ppm of the test substance to 50 F344 rats and 50 B6C3F$_1$ mice of either sex for 103 weeks. Groups of 50 untreated rats and mice of either sex served as controls.

In the chronic bioassay, mean body weights of dosed and control rats of either sex were comparable over the course of the study. Feed consumption by low- and high-dose male rats was 92% and 95% that of the controls, and feed consumption by low- and high-dose female rats was 87% and 79% that of the controls. Mean body weights of high-dose mice of either sex were lower than those of controls; feed consumption by dosed mice was comparable with that of controls. Although the rats and mice might have been able to tolerate higher doses, 50,000 ppm (5%) is the recommended maximum concentration of a test substance mixed in feed, according to the guidelines of the Bioassay Program.

No tumors were observed in increased incidences that were considered to be related to administration of tara gum to either species. Interstitial cell tumors of the testis in male rats were observed in a statistically significant ($P \leq 0.003$ for trend and group comparisons) positive relationship (40/48 controls; 46/46 low dose; 48/48 high dose); because these tumors are present in almost all aged F344 male rats and because of the marginal statistical significance when time-adjusted analyses are applied, these increases are not regarded as being related to tara gum administration.

A significant ($P<0.05$) negative trend was observed in the proportion of male rats with pancreatic islet cell adenoma (5/45 controls, 1/44 low dose, 0/45 high dose), of female mice with alveolar/bronchiolar adenomas (7/50, 2/49, 2/50), and of female mice with hepatocellular adenomas (9/49, 4/49, 1/50).

Under the conditions of this bioassay, tara gum was not carcinogenic for F344 rats or B6C3F$_1$ mice of either sex.

Report Date: March 1982

TR-225 Carcinogenesis Bioassay of D & C Red No. 9 (CAS No. 5160-02-1) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of D & C Red No. 9, a pigment used in topical drugs and cosmetics, was conducted by feeding diets containing 1,000 or 3,000 ppm of the test substance (89.8% pure) to groups of 50 F344 rats of either sex for 103 weeks. Similar groups of 50 B6C3F$_1$ mice received diets containing 1,000 or 2,000 ppm of the test substance for 103 weeks. Groups of 50 untreated rats and mice of either sex served as controls.

In a 13-week subchronic study, the spleens of most dosed rats were enlarged and pigment (unidentified) was present in the renal tubular epithelium. Lymphoreticular hyperplasia of thymic lymph nodes was found in 75-100% of females receiving 6,000-50,000 ppm D & C Red No. 9 and in 70-100% of male rats receiving 3,000-25,000 ppm. Hemosiderosis of the liver was observed at the high-dose levels in male and female rats. Mice receiving 1,250 ppm or more D & C Red No. 9 had congestion of the spleen and hemosiderin deposits. Thus, the selection of doses for the chronic study was based on the appearance of hemosiderosis and the incidences and severity of splenic lesions observed in the 91-day subchronic study.

In the chronic study, mean body weights of dosed rats of either sex and of male mice were comparable with those of controls. After week 50, the mean body weight of high-dose female mice was lower than that of the controls. No compound-related effects on survival or clinical signs were observed for rats or mice of either sex. With the possible exception of female mice, all other dosed groups of rats or mice might have tolerated higher doses, thus a clear maximum tolerated dose may not have been utilized in this study.

Splenic sarcomas (0/50, 0/50, 26/48; $P<0.001$) and neoplastic nodules of the liver (0/50, 6/50, 7/49; $P<0.01$) were observed in high-dose male rats at incidences significantly higher than those in the controls. Incidences of neoplastic nodules in the livers (1/50, 1/50, 5/50) of female rats showed a statistically significant ($P<0.05$) trend. Nonneoplastic splenic lesions were also observed in dosed male and female rats.

Lymphocytic leukemia was observed in dosed male (10/50, 2/50, 2/50) and female (10/50, 2/50, 1/50) rats at statistically significant ($P<0.05$) decreased incidences, compared with controls. Adenomas or carcinomas of the preputial gland in male rats (7/50, 2/50, 0/50) occurred with a statistically significant ($P<0.01$) negative relationship to dose of D & C Red No. 9 ($P=0.007$).

Under the conditions of this bioassay, D & C Red No. 9 was carcinogenic for male F344 rats causing an increased incidence of sarcomas of the spleen and a dose-related increase in neoplastic nodules of the liver. D & C Red No. 9 was not considered to be carcinogenic to female F344 rats, although the increased incidence of neoplastic nodules of the liver may have been associated with administration of the test chemical. D & C Red No. 9 was not carcinogenic for B6C3F$_1$ mice of either sex.

Synonyms: 5-chloro-2-[(2-hydroxy-1-naphthalenyl) azo]-4-methylbenzene sulfonic acid, barium salt; C.I. Pigment Red; C.I. Pigment Red 53:1; C.I. Pigment Red, barium salt

Report date: May 1982

TR-226 Carcinogenesis Bioassay of C.I. Solvent Yellow 14 (CAS No. 842-07-9) in F344/N Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of C.I. Solvent Yellow 14 (94.1% pure), a widely used monoazo dye, was conducted by feeding diets containing 250 or 500 ppm of C.I. Solvent Yellow 14 to groups of 50 F344 rats of either sex for 103 weeks. Similar groups of 50 B6C3F$_1$ mice received diets containing 500 or 1,000 ppm of C.I. Solvent Yellow 14 for 103 weeks. Groups of 50 untreated rats and mice of either sex served as controls.

Throughout the bioassay, mean body weights of dosed rats and mice were slightly lower than those of controls. No compound-related clinical signs or effects on survival were observed.

Increases in nonneoplastic lesions included cardiac valve fibrosis for male and female rats, lymphoid hyperplasia of the lung for male rats, and for female rats, bile duct hyperplasia, focal atrophy of the pancreatic acinus, and nephropathy. None of these effects were observed in mice.

Neoplastic nodules of the liver occurred in rats of either sex with a dose-related trend that was significant (male, $P<0.001$; female, $P=0.005$), and the incidences in the high-dose groups were significantly higher than those in the controls (male: control, 5/50; low-dose, 10/50; high-dose, 30/50, $P<0.001$ and female: control, 2/50; low-dose, 3/49; high-dose, 10/48, $P=0.011$).

Lymphomas or leukemias occurred in low-dose female mice at an incidence significantly ($P<0.05$) higher than that in the controls (12/50, 23/50, 17/50). Because of the lack of a dose-related trend and because the incidence in the high-dose group was not significant, the association between the increased incidence of hematopoietic tumors in the low-dose group and the administration of C.I. Solvent Yellow 14 is not clearly established. The incidence of lymphomas or leukemias in male mice was higher (not statistically significant) than that in the corresponding controls (5/49, 10/50, 10/50); in both low- and high-dose rats of either sex the incidence was significantly ($P<0.001$) lower than that in controls.

Under the conditions of this bioassay, C.I. Solvent Yellow 14 was carcinogenic in male and female F344/N rats, as evidenced by increased incidences of neoplastic nodules of the liver. C.I. Solvent Yellow 14 was not carcinogenic for B6C3F$_1$ mice of either sex.

Synonym: 1-(phenylazo)-2-naphthol

Report Date: September 1982

TR-227 Carcinogenesis Bioassay of Gum Arabic (CAS No. 9000-01-5) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of gum arabic (81-86% pure), a widely used food stabilizer, was conducted by feeding diets containing 25,000 or 50,000 ppm of the test substance to 50 F344 rats and 50 B6C3F$_1$ mice of each sex for 103 weeks. Groups of untreated rats and mice of each sex served as controls.

Throughout most of the study, mean body weights of dosed male and female mice and of dosed male rats were comparable with those of the controls; mean body weights of the dosed female rats were slightly lower than those of the controls. No other compound-related clinical signs or effects on survival were observed. Mean daily feed consumption by high-dose rats and mice of either sex was 85% to 94% that of the controls. The high dose (50,000 ppm) used in this bioassay is the maximum concentration (5%) currently used in feed studies.

Statistically significant ($P<0.05$) increasing trends were observed for the number of female mice with hepatocellular carcinomas (1/49, 2/50, 6/50), and with total liver tumors (4/49, 2/50, 10/50). No statistically significant differences were obtained when comparing the control rates with those observed in the treated groups. These observations were not considered to be clearly associated with the dietary administration of gum arabic. Thus, no compound-related neoplastic or non-neoplastic lesions were found in rats or mice of either sex.

Under the conditions of this bioassay, gum arabic was not carcinogenic for F344 rats or B6C3F$_1$ mice of either sex.

Synonym: gum acacia

Report Date: May 1982

TR-228 Carcinogenesis Bioassay of Vinylidene Chloride (CAS: 75-35-4) in F344 Rats and B6C3F$_1$ Mice (Gavage Study)

A subchronic and a chronic carcinogenesis study of vinylidene chloride (99% pure), a widely used chemical intermediate and monomer, were conducted in F344/N rats and B6C3F$_1$/N mice. In subchronic studies, groups of 10 rats and 10 mice of either sex were administered vinylidene chloride in corn oil by gavage five times per week at 0, 5, 15, 50, 100, or 250 mg/kg body weight for 13 weeks. At the end of this study, representative tissues from these animals were subjected to histopathological examination. The liver was identified as a target organ for vinylidene chloride toxicity.

In the 104-week chronic exposure study, conducted primarily to determine possible carcinogenic potential of vinylidene chloride by the oral route, 50 F344/N rats and 50 B6C3F$_1$/N mice of either sex were gavaged with vinylidene chloride suspended in corn oil at dose levels of 1 or 5 mg/kg (rats) and 2 or 10 mg/kg (mice). Groups of 50 rats and 50 mice of either sex received corn oil alone and served as vehicle controls.

Throughout most of the study, mean body weights of the dosed rats of either sex and high-dose female mice were slightly lower than those of the controls. The absence of compound-related effects on survival or clinical signs suggests that the rats and mice of either sex could have tolerated higher doses. While no significant differences in survival were observed for any group of rats, 12 control and 10 low-dose males were killed accidentally during week 82; this may have compromised the sensitivity of the male rats study.

The results of histopathological examination indicated an increased incidence of necrosis of the liver in high-dose male and low-dose female mice and chronic renal inflammation in high-dose rats of either sex.

The only observed significant ($P>0.05$) increase in tumor incidence occurred in low-dose female mice: lymphoma (2/48, 9/49, 6/50) and lymphoma or leukemia (7/48, 15/49, 7/50). These increases were not considered to be related to vinylidene chloride administration because similar effects were not found in the high-dose female mice or in male mice or rats.

Under the conditions of this bioassay, vinylidene chloride administered by gavage was not carcinogenic for F344/N rats or B6C3F$_1$/N mice of either sex. However, since the use of a maximum tolerated dose in this study has not been clearly demonstrated and since previously reported studies have shown that carcinogenicity is associated with inhalation exposure to vinylidene chloride, this study should not be taken as proof that the chemical is not a carcinogen.

Report Date: May 1982

Synonyms: 1,1-dichloroethylene; VDC; 1,1-DCE

TR-229 Carcinogenesis Bioassay of Guar Gum (CAS No. 9000-30-0) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of guar gum, a widely used food stabilizer, was conducted by feeding diets containing 25,000 or 50,000 ppm of the test substance from two batches having purities of 83.5% and 91.9% to 50 F344 rats and 50 B6C3F$_1$ mice of either sex for 103 weeks. Groups of 50 untreated rats and mice of either sex served as controls. The rodents might have tolerated higher doses but 50,000 ppm (5% of diet) is the upper limit for chronic feeding studies in the Bioassay Program, and this level represented the maximum tolerated dose (MTD) for females of both species in the present study.

After week 20 in mice and week 40 in rats, mean body weights of high-dose females were lower than those of the untreated controls. No compound-related clinical signs or adverse effects on survival were observed. Feed consumption by dosed rats and dosed mice of either sex was lower than that of the controls. There were increased incidences of adenomas of the pituitary (8/45, 18% controls; 17/46, 37% low dose; 17/43, 40% high dose) in male rats and phe-

ochromocytomas of the adrenal (0/50, 0%; 5/50, 10%; 6/50, 12%) in female rats, but these differences ($P<0.035$) were considered to be unrelated to administration of guar gum. When pituitary adenomas or carcinomas and when pheochromocytomas or malignant pheochromocytomas are combined, the statistical differences disappear.

Hepatocellular carcinomas (15/44, 34%; 6/50, 12%; 6/49, 12%) occurred in treated male mice at incidences significantly ($P \leq 0.011$) lower than that in controls. The combined incidence of male mice with either hepatocellular adenomas or carcinomas (16/44, 36%; 12/50, 24%; 7/49, 14%) was also significantly ($P = 0.013$) lower in the high-dose group.

Under the conditions of this bioassay, guar gum was not carcinogenic for F344 rats or B6C3F$_1$ mice of either sex.

Report Date: March 1982

TR-230 Carcinogenesis Bioassay of Agar (CAS No. 9002-18-0) in F344 Rats and B6C3F$_1$ Mice (Feed Study)

A carcinogenesis bioassay of agar isolated from *Pterocladia*, a gelling agent used in foods and pharmaceuticals, was conducted on groups of 50 F344 rats and 50 B6C3F$_1$ mice of either sex which were fed diets containing 25,000 or 50,000 ppm of the test substance for 103 weeks. Groups of 50 untreated rats and mice of either sex served as controls.

Mean body weights of dosed and control male rats were comparable throughout the study. After week 80, mean body weights of dosed female rats were slightly lower than those of the controls. Mean body weights of dosed and control male mice were comparable throughout the study. The mean body weights of dosed female mice were lower than those of the controls at week 20 and remained lower throughout the study. No compound-related effects on survival, feed consumption, clinical signs of toxicity, or tumor incidence were observed. Although the rats of either sex and male mice might have been able to tolerate higher doses, 50,000 ppm was the administered high-dose level since that is the maximum concentration of a test substance in feed recommended in the guidelines of the Bioassay Program.

A statistically significant trend ($P = 0.015$) was observed for the increased incidence of cortical adenomas of the adrenal gland (control, 0/50; low-dose, 0/50; high-dose, 4/50) in female rats; the difference between control and high-dose groups was not significant. In male mice the incidence of hepatocellular adenomas (control, 0/49; low-dose, 3/50; high-dose, 7/50) was significantly ($P = 0.007$) increased in the high-dose group when compared with controls; likewise, the overall trend was significant ($P = 0.005$). The incidence of total liver tumors (control, 9/49; low-dose, 8/50; high-dose, 13/50) did not differ significantly among the groups. Neither of these increases (in cortical adenomas or in liver tumors) was considered to be compound related.

Under the conditions of this bioassay, the agar isolated from *Pterocladia* was not carcinogenic for F344 rats or B6C3F$_1$ mice of either sex.

Report Date: March 1982

TR-231 Carcinogenesis Bioassay of Stannous Chloride (CAS No. 7772-99-8) in F344/N Rats and B6C3F$_1$/N Mice (Feed Study)

Stannous chloride is an inorganic tin compound used as a food preservative, a stabilizer for colors, perfumes, and soaps, and as a reducing agent in tin plating. It is also used as a mordant in printing, a silvering agent for glass and plastics, a catalyst for curing phenolic resins, an additive to drilling muds, and an antisludge agent for oils.

The chronic phase of a carcinogenesis bioassay for stannous chloride was conducted by feeding diets containing 1,000 or 2,000 ppm stannous chloride to groups of 50 F344/N rats and 50 B6C3F$_1$/N mice of each sex for 105 weeks. Similar groups of untreated rats and mice served as controls.

In this study, the concentrations of tin in bone, kidney, and liver were no higher than those attained in other lifetime studies utilizing 1/100 of the dose, suggesting that organ accumulation of tin was not dose dependent, but probably limited by absorption.

Mean body weight gain and feed consumption of dosed and control rats and mice were comparable. Survival of high-dose male rats was somewhat lower than that of the control and low-dose groups (37/50, control; 39/50, low-dose; 30/50, high-dose). Survival of control male mice was less ($P<0.05$) than that of either dosed group (32/50, 42/50, 45/50); survival of the female mice appeared to be dose related (38/50, 33/50, 28/50).

C-cell adenomas of the thyroid (2/50, 9/49, 5/50), C-cell adenomas or carcinomas combined (2/50, 13/49, 8/50), and adenomas of the lung (0/50, 0/50, 3/50) in male rats; and hepatocellular carcinomas or adenomas combined (3/49, 4/49, 8/49) and histiocytic malignant lymphomas (0/50, 0/49, 4/49) in female mice occurred with significant ($P<0.05$) positive trends and/or with significantly ($P<0.05$) increased incidences in the dosed groups when compared with the paired controls. However, when the lung adenomas in male rats are combined with lung carcinomas and when all lymphomas in female mice are considered, no statistical significance remains. For the thyroid C-cell tumors in male rats and for the liver tumors in female mice, the incidences in the high-dose groups were not significantly different from the historical control rates at that laboratory (C-cell tumors: 32/288, 11.1%; liver tumors: 24/297, 8%). When the historical control rate is used as a basis for comparison, the low-dose incidence of thyroid C-cell tumors remains significant ($P<0.01$).

Under the conditions of this bioassay, stannous chloride was judged not to be carcinogenic for male or female F344/N rats or B6C3F$_1$/N mice, although C-cell tumors

of the thyroid gland in male rats may have been associated with the administration of the test chemical.

Report Date: June 1982

TR-232 Carcinogenesis Studies of Pentachloroethane (CAS: 76-01-7) in F344/ N Rats and B6C3F$_1$ Mice (Gavage Study)

A carcinogenesis bioassay of technical grade pentachloroethane (95.5% pure, with 4.2% hexachloroethane) was conducted by administering the test chemical in corn oil by gavage to groups of 50 male and 50 female F344/ N rats at doses of 75 or 150 mg/ kg body weight and to groups of 50 male and 50 female B6C3F$_1$ mice at doses of 250 or 500 mg/ kg. (Pentachloroethane is a solvent that was used primarily as an intermediate in the manufacture of tetrachloroethylene.) Doses were administered for 103 weeks for rats and 41-103 weeks for mice. Groups of 50 rats and 50 mice of each sex received corn oil by gavage on the same dosing schedule and served as vehicle controls. Prechronic testing (single-dose and 14-day and 13-week repeated-dose studies) did not indicate target organ toxicity for pentachloroethane. The dosage levels for the 2-year study were selected on the basis of survival and body weight gains during the prechronic test phase.

Survival of high-dose rats of each sex was significantly ($P<0.05$) less than that of the controls. Mean body weights of dosed male and female rats were lower than those of the corresponding controls during the second year of the study. Final mean body weights for rats were 4%-5% lower for male rats and 8%-12% lower for female rats when compared to controls.

Chronic, diffuse inflammation of the kidney, distinguishable from nephropathy seen in aging F344/ N rats, was found in male rats in a significant ($P<0.001$) and dose-related incidence (control, 4/50, 8%; low-dose, 14/49, 29%; high-dose, 33/50, 66%). Mineralization of the renal papilla, considered to be secondary to chronic inflammation, was also observed at increased incidences in dosed male rats.

Pentachloroethane administration did not cause any increased incidences of tumors in either male or female rats. [See Note Added Subsequent to Peer Review.] Statistically significant negative trends were detected for subcutaneous tissue fibromas among males and for pituitary adenomas in both sexes.

Forty-two high-dose male mice died by week 41, and the 8 remaining animals in the group were killed at that time. Twenty-five male control mice were killed at week 44 to serve as controls for the high-dose males. Only 22/50 (44%) of the low-dose male mice survived to the end of the study. All high-dose female mice were dead by week 74, and only 9/50 (18%) low-dose females survived to the end of the study. Mean body weights of mice were lower than those of controls.

The incidence of hepatocellular carcinoma was significantly elevated in all groups of dosed mice (male: 4/48, 8%; 26/44, 59%, $P<0.001$; 7/45,16%; female: 1/46, 2%; 28/42, 67%, $P<0.001$;13/45, 29% $P<0.001$). Early mortalities in the high-dose male mice precluded an evaluation of their lifetime incidence of hepatocellular carcinoma. There was a significant increase in incidence over that observed among 25 controls killed at week 44 (0/25 versus 7/45, $P<0.05$). There was also a significant ($P<0.001$) dose-related increase in hepatocellular adenoma in female mice (2/46, 4%; 8/42, 19%; 19/45, 42%).

Under the conditions of this bioassay, technical grade pentachloroethane containing 4.2% hexachloroethane (a known carcinogen in mice) was not carcinogenic in F344/ N rats. The decreased survival of dosed rats might have reduced the sensitivity for a carcinogenic response in this species. Pentachloroethane was nephrotoxic to male rats. Technical grade pentachloroethane was carcinogenic for B6C3F$_1$ mice, causing hepatocellular carcinomas in males and females, and adenomas in females.

NOTE ADDED SUBSEQUENT TO PEER REVIEW

After the Peer Review Panel meeting in June 1981, the National Toxicology Program determined that the kidney (especially in male F344/N rats) was a target organ for the short-chain chlorinated aliphatic hydrocarbons. This awareness came from the nonneoplastic and neoplastic diagnoses made on related chemicals in this class. Alerted to this lead, the NTP re-examined the originally-prepared histology slides on the rat kidney from the pentachloroethane bioassay. During the re-reading, additional renal tubular adenomas were discovered. Unfortunately, these slides were lost after they arrived at the Gulf South Research Institute laboratory; by necessity, a new set of slides was prepared.

In the second set of slides, three additional renal tubular-cell adenomas were discovered: one in a low-dose male and two in high-dose males; none were found in treated females or in male and female vehicle controls. Thus, rare tubular-cell adenomas of the kidney occurred in male rats with a dose-related trend ($P<0.05$), and the incidence in the high-dose group was suggestive ($P<0.06$; 0/50, 1/49, 4/50). Additionally, one control and one low-dose male each had an adenocarcinoma and another low-dose male had a carcinoma of the kidney (not otherwise specified); combining tubular-cell tumors reduced the statistical differences (1/50, 2/49, 4/50). These tumors are uncommon in male vehicle controls in the bioassay program, occurring in 1/293 (0.3%) at this bioassay testing laboratory and in 4/998 (0.4%) in all NCI/ NTP bioassay testing laboratories. All tumors in these gavage controls were adenocarcinomas. The National Toxicology Program considers that these rare tubular-cell tumors of the kidney in male rats indicate a target organ and may have been associated with the administration of pentachloroethane. These additional tumor diagnoses were not presented to the Peer Review Panel. These are, however, the incidence rates recorded and analyzed statistically in this technical report (See Table 5, Table A1, and Table A3 in the full document).

Report Date: April 1983

Synonyms: pentalin

TR-233 Carcinogenesis Bioassay of 2-Biphenylamine Hydrochloride (CAS No. 2185-92-4) in F344/N Rats and B6C3F$_1$ Mice (Feed Study)

2-Biphenylamine (2-aminobiphenyl) is a chemical intermediate used in the manufacture of C.I. Acid Red 15. It is present as a contaminant in 4-biphenylamine (a rubber antioxidant) and in diphenylamine (a dye intermediate, stabilizer for nitrocellulose explosives, and a topical agent for prevention of screwworm infestation in animals).

Single-dose, 14-day, and 13-week studies were conducted using technical-grade 2-biphenylamine (2-aminobiphenyl) containing up to 2.5% of the carcinogenic contaminant, 4-biphenylamine. When the contamination was recognized, analytical development was begun to purify the material. The salt, 2-biphenylamine hydrochloride, was prepared to obtain a more pure test product, which contained 0.006%-0.049% 4-biphenylamine. The prechronic tests were completed by the time purification was accomplished, so data from a second 14-day study with 2-biphenylamine hydrochloride were used to help set dose levels for the chronic study.

The results of the comparative 14-day studies showed that technical-grade 2-biphenylamine was more toxic to mice than rats than 2-biphenylamine hydrochloride as evidenced by greater incidence of splenomegaly and greater weight gain depression.

The technical-grade 2-biphenylamine caused a dose-related decrease in hemoglobin concentration and a dose-related increase in leucocyte count in male and female mice in the 13-week study. Hemosiderosis, congestion, and extramedullary hematopoiesis were present in the spleens of nearly all rats receiving 3,000 ppm or more of the chemical, and in nearly all mice with 1,000 ppm or more 2-biphenylamine in their diets.

The chronic study was conducted with the purified 2-biphenylamine hydrochloride by feeding diets containing 1,000 or 3,000 ppm 2-biphenylamine hydrochloride to groups of 49 or 50 F344/N rats and 50 B6C3F$_1$ mice of each sex for 103 weeks. Groups of 50 rats and 50 mice of each sex served as controls. Survival of dosed male and female rats and dosed female mice was comparable with that of the corresponding controls. Survival of high-dose male mice was significantly ($P<0.010$) less than that of low-dose and control male mice.

There were little or no differences in body weight changes for rats or mice between dosed and control groups, although there was a slight decrease in body weight gain at the end of the study for high-dose male (-11%) and female (-8%) rats.

Inflammatory cells and interstitial fibrosis were found in increased incidence in the kidneys of dosed male rats as compared with controls and were considered to be compound related. In addition to the increase in renal inflammation and fibrosis, dosed male rats had more focal cellular changes of the liver than did the controls. There were no increased or decreased incidences of tumors in rats that could be associated with chemical administration.

Myelomonocytic leukemia in male rats (control, 14/50; low-dose, 1/50; high-dose, 4/50) and fibroadenomas of the mammary gland in female rats (22/50, 10/49, 9/50) occurred with significantly ($P<0.03$) decreasing trends and the incidences in the dosed groups were significantly ($P<0.02$) lower than that in the controls. There were no increased or decreased incidences of tumors in rats that could be associated with chemical administration.

Hemangiosarcomas from all sites occurred in female mice with a statistically significant ($P\leq 0.002$) positive trend. The observed incidence of hemangiosarcomas was 0/49, 1/50, and 7/50 in the control, low-dose, and high-dose groups, respectively. The incidence in the high-dose group was significantly ($P<0.01$) higher than that in controls. The conclusion that this was due to 2-biphenylamine rather than the contaminant, 4-biphenylamine, is supported by the absence of urinary bladder tumors, which are common to 4-biphenylamine.

Hemangiosarcomas also occurred in male mice with a statistically significant positive trend ($P=0.040$ by a life table test), with incidences of 0/50, 2/50, and 3/50. None of the pairwise comparisons were statistically different. The development of hemangiosarcomas may have been curtailed in the high-dose group of male mice, since only 21/50 survived until the termination of the study. The hemangiosarcomas found in female mice are uncommon with only 6/816 (0.7%) previously seen in controls at the same laboratory. The rate for control male mice is equally low: 7/803 (0.9%).

Alveolar/bronchiolar adenomas of the lung occurred at a significantly ($P<0.01$) decreased rate in male mice with an incidence in dose groups lower ($P<0.05$) than that in controls.

Under the conditions of the bioassay, 2-biphenylamine hydrochloride was not carcinogenic for F344/N rats of either sex. 2-Biphenylamine hydrochloride was carcinogenic for B6C3F$_1$ female mice, inducing hemangiosarcomas at various sites. The evidence for an association between the administration of 2-biphenylamine hydrochloride and the increased incidence of hemangiosarcomas in male mice was equivocal.

Report Date: October 1982

TR-234 Carcinogenesis Bioassay of Allyl Isothiocyanate (CAS No. 57-06-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Study)

A 2-year carcinogenesis bioassay of food-grade allyl isothiocyanate (greater than 93% purity), a flavoring agent, was conducted by administering 12 or 25 mg/kg allyl isothiocyanate in corn oil five times per week by gavage to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex for 103 weeks. Groups of 50 rats and 50 mice of each sex received corn oil alone and served as vehicle controls.

A single-dose study, a 14-day study, and a 13-week study were performed before the chronic study was conducted. Pathologic findings seen in the 14-day study at 50 mg/kg included a thickened mucosal surface of the stomach in rats and mice and a thickened urinary bladder wall in male mice. No gross or microscopic lesions were seen at the highest dose level (25 mg/kg) in the 13-week study.

In the chronic study, survival of the dosed and control rats of each sex was comparable. Throughout the study, the mean body weights of high-dose male rats were lower than those of the controls, while during the last half of the study the mean body weights of the low-dose and high-dose female rats were higher than the mean body weights of the control animals. Final body weights in control and dosed groups were comparable.

Transitional-cell papillomas in the urinary bladder occurred in dosed male rats with a statistically significant trend ($P<0.05$; controls, 0/49, 0%; low-dose, 2/49, 4%; high-dose, 4/49, 8%). This tumor has not been observed among 568 untreated male control F344/n rats at this laboratory. The incidence of transitional papillomas in male vehicle control rats in all laboratories in the NCI/NTP Bioassay Program is 1/994 (0.1%). Epithelial hyperplasia in the urinary bladder was also observed at increased incidences in dosed male rats (0/49, 1/49, 6/49). The hyperplasia did not occur in the same animals that had papillomas.

Fibrosarcomas in the subcutaneous tissue occurred in female rats with a statistically positive trend ($P<0.05$; controls, 0/50, 0%; low-dose, 2/49, 4%; high-dose, 4/49, 8%). This tumor has not been observed among 568 untreated male control F344/N rats at this laboratory. The incidence of transitional- cell papillomas in male vehicle control rats in all laboratories in the NCI/NTP Bioassay Program is 1/994 (0.1%). Epithelial hyperplasia in the urinary bladder was also observed at increased incidences in dosed male rats (0/49, 1/49, 6/49). The hyperplasia did not occur in the same animals that had papillomas.

Fibrosarcomas in the subcutaneous tissue occurred in female rats with a statistically significant positive trend ($P<0.05$; controls, 0/50, 0%; low-dose, 0/50, 0%; high-dose, 3/50, 6%), but the incidence in the high-dose group was not significant when compared with that in the control group. The historical incidence of this lesion is 1/591 (0.2%) in untreated control female F344/N rats at this laboratory and 9/999 (0.9%) in female gavage control rats in all laboratories in the Bioassay Program.

Survival of control and dosed female mice, although comparable, was unusually low. Mean body weights of high-dose mice of each sex were higher than those of the controls throughout most of the study. Final body weights in the control and dosed groups were comparable. The mice probably did not receive the maximum tolerated dose of allyl isothiocyanate.

The increased incidence of cytoplasmic vacuolization in the liver of dosed male mice was related to administration of allyl isothiocyanate (controls, 2/49, 4%; low-dose, 8/49, 16%; high-dose, 13/50, 26%).

Under the conditions of this bioassay, allyl isothiocyanate was carcinogenic for male F344/N rats, causing transitional-cell papillomas in the urinary bladder. Evidence for associating allyl isothiocyanate with subcutaneous fibrosarcomas in female F344/N rats was equivocal. Allyl isothiocyanate with subcutaneous fibrosarcomas in female F344/N rats was equivocal. Allyl isothiocyanate was not carcinogenic for $B6C3F_1$ mice of either sex.

Report Date: October 1982

TR-235 Carcinogenesis Bioassay of Zearalenone (CAS No. 17924-92-4) in F344/N Rats and $B6C3F_1$ Mice (Feed Study)

A carcinogenesis bioassay of zearalenone, an estrogenic mycotoxin, was conducted by feeding diets containing 25 or 50 ppm zearalenone to groups of 50 F344/N rats of each sex and 50 or 100 ppm to groups of 50 $B6C3F_1$ mice of each sex for 103 weeks. Groups of 50 rats and 50 mice of each sex served as controls. Estimates based on food consumption data indicate that the low- and high-dose rats received daily doses of about 1 and 2 mg, respectively, of zearalenone per kg of body weight. Low-dose and high-dose mice received estimated daily doses of about 7-10 and 14-20 mg, respectively, of zearalenone per kg of body weight.

Survival of dosed and control rats of each sex was comparable. Mean body weight gains of dosed rats of each sex were lower than those of the corresponding controls; depression in mean body weight gain was dose related. Final body weights of dosed rats were <9% lower than those of control rats. The average daily feed consumption of dosed rats of each sex was 91%-102% that of controls.

Inflammation of the prostate, testicular atrophy, and hepatocellular cytoplasmic vacuolization (male rats), and nephrosis (male and female rats) were compound-related. Retinopathy and cataracts occurred in low- and high-dose male rats and in low-dose female rats, and were associated with the closeness to fluorescent light. No compound-related, increased tumor incidences were observed in rats in the chronic study.

Survival of dosed and control mice of each sex was comparable. Mean body weight gains of high-dose male and low-dose female mice were lower than those of the controls. Terminal body weights of dosed mice were <8% below those of control mice. The average daily feed consumption by dosed mice of each sex was 97-99% that of the controls.

Myelofibrosis in the bone marrow, uterine fibrosis, and cystic ducts in the mammary gland were related to the administration of zearalenone in female mice. The incidence of hepatocellular adenomas in female mice was dose related ($P \leq 0.003$), and the incidence of these tumors in high-dose female mice was significantly higher ($P \leq 0.006$) than those in the controls (control, 0/50; low-dose, 2/49; high-dose, 7/49). Pituitary adenomas occurred with

statistically significant positive trends ($P \leq 0.022$ for males and $P \leq 0.001$ for females). The incidences of these tumors in high-dose mice were significantly increased relative to controls ($P \leq 0.032$ for males: 0/40, 4/45, 6/44; and $P \leq 0.003$ for females: 3/46, 2/43, 13/42).

Under the conditions of this bioassay, zearalenone was not carcinogenic for F344/N rats of either sex. Zearalenone should be considered carcinogenic in $B6C3F_1$ mice, as evidenced by the increased proportion of male and female mice with pituitary adenomas and by the increased proportion of female mice with hepatocellular adenomas.

Synonym: trans-zearalenone

Report Date: October 1982

TR-236 Carcinogenesis Bioassay of D-Mannitol (CAS No. 69-65-8) in F344/N Rats and $B6C3F_1$ Mice (Feed Study)

A carcinogenesis bioassay of D-mannitol (98%-100% pure), a food and drug additive, was conducted by feeding diets containing 25,000 or 50,000 ppm D-mannitol to groups of 50 F344/N rats and 50 $B6C3F_1$ mice of each sex for 103 weeks. Groups of 50 rats and 50 mice of each sex served as controls.

Survival and mean body weights of dosed and control male rats and of dosed and control mice of each sex were comparable. Survival of high-dose female rats was significantly higher ($P<0.05$) than that of the low-dose female rats. However, neither the survival of the low-dose group nor that of the high-dose group was significantly different from that of the controls. Throughout the study, mean body weight gain of dosed female rats was depressed ($\leq$ 10%) relative to that of controls. Feed consumption by dosed and control rats and mice of each sex was similar.

Although the rats and mice of each sex might have been able to tolerate higher doses, a dietary level of 50,000 ppm (5%) is the maximum concentration of a test substance in feed recommended in the guidelines of the Bioassay Program.

Dilatation of the gastric fundal gland was observed in the increased incidence in dosed female rats when compared with that of controls (control, 6/50, 12%; low dose, 23/50, 46%; high dose, 23/50, 46%). Retinopathy and cataracts occurred at increased incidences in high-dose male and low- and high-dose female rats.

A mild nephrosis, characterized by focal vacuolization of the renal tubular epithelium, was observed in increased incidence in dosed mice of each sex and was considered to be related to administration of D-mannitol (males: control, 15/50, 30%; low dose, 29/50, 58%; high dose, 30/47, 64%; females: control, 1/48, 2%; low dose, 3/48, 6%; high dose, 14/49, 29%).

Under the conditions of this bioassay, D-mannitol was not carcinogenic for F344/N rats or $B6C3F_1$ mice of either sex.

Report Date: September 1982

TR-237 Carcinogenesis Studies of 1,1,1,2-Tetrachloroethane (CAS: 630-20-6) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Carcinogenesis studies of technical grade 1,1,1,2-tetrachloroethane (>99% pure) were conducted by administering the test chemical in corn oil by gavage to groups of 50 male and 50 female F344/N rats at doses of 125 or 250 mg/kg body weight and to groups of 50 male and 50 female $B6C3F_1$ mice at doses of 250 or 500 mg/kg. (1,1,1,2-tetrachloroethane is used as a chemical intermediate in the production of trichloroethylene and tetrachloroethylene). Doses were administered five times per week for 103 weeks. Due to chemically induced toxicity, high-dose mice received the chemical for only 65 weeks. Groups of 50 rats and 50 mice of each sex received corn oil by gavage on the same dosing schedule and served as vehicle controls.

The chemical produced cumulative toxic effects with signs of central nervous system involvement from week 44 forward in the chronic study, resulting in significantly lower survival of high-dose male rats ($P=0.001$), and possibly decreasing the incidence of late-developing tumors in this group. Mean body weights of dosed and control rats of each sex were similar. Fourteen control, 10 low-dose, and 3 high-dose male rats and 2 control, 5 low-dose, and 8 high-dose female rats were killed accidentally during the study; of these, 11 control and 7 low-dose males died apparently from heat stress during week 62 as a result of a 6-hour elevated temperature (>34°C) in the animal room.

Neither hepatocellular neoplastic nodules alone nor hepatocellular carcinomas alone occurred in statistically significant incidences in male rats, but the combined incidence of male rats with either hepatocellular neoplastic nodules or carcinomas occurred with a statistically significant positive trend ($P<0.05$) in the life table test (controls, 0/49, 0%; low-dose, 1/49, 2%; high-dose, 3/48, 6%). A single hepatocellular carcinoma occurred in the high-dose group. The combined incidence of liver tumors in the high-dose males (3/48, 6%) did not greatly exceed the historical incidences of liver tumors in groups of vehicle controls in other studies at this laboratory (5/243, 2.1%; range 0%-4%). However, reduced survival of the high-dose group in the present study may have reduced the sensitivity of this bioassay for detecting liver tumors. Mineralization of the kidney increased in a dose-related fashion in the male rats (12/48, 19/50, 26/48).

Fibroadenomas in the mammary gland of female rats occurred with a statistically significant ($P<0.05$) increased incidence in the low-dose group as compared with the controls (6/49, 15/49, 7/46). The incidence in the high-dose group was not different than that in the controls.

The combined incidence of adenomas, adenocarcinomas, and carcinomas in the pituitary gland of female rats showed a statistically significant ($P<0.05$) negative trend and the incidence in the high dose group was significantly ($P<0.05$) less than that in the controls (18/39, 16/45, 7/42).

Mean body weight of high-dose mice was less than that of controls after week 20 in males and after week 40 in females. Clinical signs of central nervous system toxicity occurred at week 51 in both sexes of high-dose mice and by week 66 they were dead or moribund and were killed. Survival of low-dose females was also significantly ($P<0.05$) less than that of controls.

The maximum tolerated dose was exceeded in high-dose mice. Inflammation, necrosis, fatty metamorphosis, and hepatocytomegaly were observed in increased incidences in the livers of high-dose male and female mice. The major neoplastic histopathological effects occurred in the liver, where dose-related statistically significant ($P<0.05$) increases in the incidence of hepatocellular adenomas occurred in both male and female mice: vehicle controls, low-, and high-dose male mice had rates of 13% (6/48), 30% (14/46), and 42%, (21/50); corresponding percentages in female mice were 8% (4/49), 17% (8/46), and 50% (24/48). Evidence for the association between 1,1,1,2-tetrachloroethane and development of hepatocellular carcinomas in mice was limited because of poor survival in the high-dose groups. Nevertheless, there was an increased incidence of hepatocellular carcinomas in female mice despite the reduced survival in the dosed groups (controls, 1/49, 2%; low-dose, 5/46, 11%; high-dose, 6/48, 13%). There was no clear effect in male mice.

Under the conditions of these studies, 1,1,1,2-tetrachloroethane was not demonstrated to be carcinogenic in F344/N rats, although the observed increase in the proportion of male rats with liver tumors may have been associated with the administration of 1,1,1,2-tetrachloroethane; accidental killing of 27 male and 15 female rats reduced the sensitivity of this bioassay for detecting a carcinogenic response. 1,1,1,2-Tetrachloroethane was carcinogenic for $B6C3F_1$ mice, causing an increased proportion of female mice with hepatocellular carcinomas and an increased proportion of male and female mice with hepatocellular adenomas; the decreased survival in high-dose male and female mice compromised the ability of this bioassay to further determine the presence or absence of a carcinogenic effect and gave clear evidence that these doses were toxic.

Report Date: May 1983

TR-238 Carcinogenesis Bioassay of Ziram (CAS No. 137-30-4) in F344/N Rats and $B6C3F_1$ Mice (Feed Study)

A carcinogenesis bioassay of ziram (89% pure, with 6.5% thiram), a fungicide and a rubber vulcanization accelerator, was conducted in F344/N rats and in $B6C3F_1$ mice. Groups of 50 rats of each sex received diets containing 300 or 600 ppm of commercial grade ziram for 103 weeks; groups of 49 or 50 mice of each sex received diets containing 600 or 1,200 ppm ziram; and groups of 50 rats and 50 mice of each sex served as untreated controls.

The average daily consumption of ziram by low- and high-dose rats, through the majority of the study, was about 11 and 22 mg/kg for males and 13 and 26 for females. The average daily consumption of ziram by low- and high-dose mice, through the majority of the study, was 122 and 196 mg/kg for males and about 131 and 248 mg/kg for females.

Survival and feed consumption and mean body weights of rats of each sex were not adversely affected by ziram; rats of each sex could have tolerated higher doses.

C-Cell carcinomas of the thyroid in male rats occurred with a statistically significant positive trend ($P<0.01$) and the incidence in the high-dose group was significantly higher ($P<0.05$) than that in the controls (control, 0/50, 0%; low-dose, 2/49, 4%; high-dose, 7/49, 14%) and higher than that previously observed in control male rats at the same laboratory (18/584, 3%; range 0% to 8%). The combined incidence of males with either C-cell adenoma or carcinoma also showed a statistically significant ($P<0.05$) positive trend (control, 4/50, 8%; low-dose, 9/49, 18%; high-dose, 12/49, 24%). There were no significant histopathologic changes noted in the follicular cells.

Survival of male and female mice was not adversely affected by ziram in feed; mean body weight gain by dosed male mice throughout the study and by high-dose female mice after week 80 was depressed by 15% to 20% relative to the controls. Average daily feed consumption by high-dose males and high-dose females was, respectively, 78% and 85% that of the controls. Mice probably could not have tolerated higher doses.

The incidence of alveolar/bronchiolar adenomas was significantly ($P<0.05$) increased in female mice (control, 2/50, 4%; low-dose, 5/49, 10%; high-dose, 10/50, 20%). The combined incidence of alveolar/bronchiolar adenomas or carcinomas in female mice showed a statistically significant ($P<0.05$) positive trend. The incidence in the high-dose group was significantly ($P<0.05$) higher than that in the controls (control, 4/50, 8%; low-dose, 6/49, 12%; high-dose, 11/50, 22%). Pulmonary adenomatous hyperplasia consistent with chronic Sendai virus infection (confirmed by serologic analysis performed on untreated animals from the same animal shipment and present in the same room) was observed in control and dosed male mice (control, 15/49, 31%; low-dose, 19/50, 38%; high-dose, 16/49, 33%) as well as in control and dosed female mice (control, 18/50, 36%; low-dose, 27/49, 55%; high-dose, 26/50, 52%). Six of the 26 high-dose females without pulmonary adenomatous hyperplasia had pulmonary tumors, whereas 4 of the 24 high-dose females without pulmonary adenomatous hyperplasia also had pulmonary tumors. Only 1 of 27 low-dose females with adenomatous hyperplasia had a pulmonary tumor.

There was a significant decrease in the incidence of mammary fibroadenomas in high-dose female rats (control, 16/50, 32%; low-dose, 17/50, 34%; high-dose, 8/50, 16%). Significant dose-related decreased incidences of liver carcinomas in male mice (control, 13/49, 27%; low-dose, 8/50, 16%; high-dose, 1/49, 2%) and of liver adenomas in female mice (control, 7/50, 14%; low-dose, 2/50, 4%; high-dose, 0/50, 0%) were observed.

Under the conditions of these studies, ziram was carcinogenic for male F344/N rats, causing increased incidences of C-cell carcinomas of the thyroid gland. Ziram

was not carcinogenic for either female F344/N rats or for male $B6C3F_1$ mice. Increased incidences of alveolar/bronchiolar adenomas and of combined alveolar/bronchiolar adenomas or carcinomas occurred in female $B6C3F_1$ mice. However, the interpretation of this increase in lung tumors is complicated by an intercurrent Sendai virus infection.

Synonym: zinc dimethyldithiocarbamate

Report Date: April 1983

TR-239 Carcinogenesis Bioassay of Bis(2-chloro-1-methylethyl)ether (~70%) (CAS No. 108-60-1) Containing 2-Chloro-1-methylethyl(2-chloropropyl)ether (~30%) (CAS No. 83270-31-9) in $B6C3F_1$ Mice (Gavage Study)

Bis(2-chloro-1-methylethyl) ether is a beta-haloether that has been used extensively in paint and varnish removers, spotting agents, and cleaning solutions. BCMEE has also been used as an intermediate in the manufacture of dyes, resins, and pharmaceuticals and has been added to soap solutions to aid in textile cleaning. Bis(2-chloro-1-methylethyl) ether has been a by-product in the manufacture of propylene oxide and propylene glycol. It is the active ingredient of a nematocide developed and used on field crops in Japan.

A carcinogenesis bioassay of bis(2-chloro-1-methylethyl)ether (~70%), containing ~30% 2-chloro-1-methylethyl(2-chloropropyl) ether, was conducted by administering 100 or 200 mg/kg bis(2-chloro-1-methylethyl)ether in corn oil by gavage 5 times per week for 103 weeks to groups of 50 $B6C3F_1$ mice of each sex. Fifty mice of each sex received corn oil alone and served as vehicle controls. Survival and mean body weights of dosed and control mice of each sex were comparable.

The incidence of alveolar/bronchiolar adenomas occurred in a positive dose-related trend for male mice ($P<0.05$: control 5/50, 10%; low-dose 13/50, 26%; high-dose 11/50, 22%) and for female mice ($P<0.02$: 1/50, 2%; 4/50, 8%; 8/50, 16%). The number of female mice in the high-dose group with adenomas was significantly ($P<0.03$) greater than that in controls. The combined incidences in dosed males and in high-dose females were significantly higher ($P\leq 0.04$ for males and $P\leq 0.01$ for females) than those in the controls (males: 6/50, 12%; 15/50, 30%; 13/50, 26%; females: 1/50, 2%; 4/50, 8%; 10/50, 20%).

The incidence of hepatocellular carcinomas (5/50, 10%;13/50, 26%; 17/50, 34%) and the combined incidence of hepatocellular adenomas and carcinomas (13/50, 26%, 23/50, 46%, 27/50, 54%) in male mice were statistically significant by the trend tests ($P<0.01$) and the incidences in the high-dose group were significantly higher than those in the controls ($P<0.01$). Metastases to the lung occurred in 1/50 control, 4/50 low-dose, 3/50 high-dose male mice. Fatty metamorphosis was found in increased incidence in the livers of dosed male mice (control 2/50; 16/50 low-dose; 15/50 high-dose).

Squamous cell papillomas were found in the stomach or forestomach in two high-dose females, one low-dose male, and one high-dose male. A squamous cell carcinoma was found in the forestomach of a third high-dose female. These tumors were probably related to administration of the test compound, since they are rarely observed in vehicle control and untreated control $B6C3F_1$ mice.

Under the conditions of this bioassay, bis(2-chloro-1-methylethyl)ether, containing 2-chloro-1-methylethyl(2-chloropropyl)ether, was carcinogenic for $B6C3F_1$ mice, causing increased incidences of alveolar/bronchiolar adenomas in male and females and hepatocellular carcinomas in males. In addition, the occurrence of a low incidence of squamous cell papillomas or carcinomas in the stomach or forestomach of females (a rare tumor in $B6C3F_1$ mice) was probably associated with the administration of bis(2-chloro-1-methylethyl)ether.

Synonyms for bis(2-chloro-1-methylethyl) ether: BCMEE; bis(2-chloroisopropyl) ether; BCPE

Report Date: December 1982

Note: Bis(2-chloro-1-methylethyl) ether was previously tested in F344 rats by gavage (See TR-191, reported 1979).

TR-240 Carcinogenesis Bioassay of Propyl Gallate (CAS No. 121-79-9) in F344/N Rats and $B6C3F_1$ Mice (Feed Study)

Propyl gallate is a white to nearly white odorless powder having a slightly bitter taste. Solutions of propyl gallate turn dark in the presence of iron or iron salts.

Propyl gallate has been used since 1948 as an antioxidant to stabilize cosmetics, food packaging materials, and foods containing fats. As an additive, it may be found in edible fats, oils, mayonnaise, shortening, baked goods, candy, dried meat, fresh pork sausage, and dried milk, and it is used in hair grooming products, pressure-sensitive adhesives, lubricating oil additives, and transforming oils.

A carcinogenesis bioassay of propyl gallate was conducted by feeding diets containing 6,000 or 12,000 ppm propyl gallate to groups of 50 F344/N rats and 50 $B6C3F_1$ mice of each sex for 103 weeks. Groups of 50 untreated rats and 50 untreated mice of each sex served as controls.

Survival of rats and mice was not adversely affected by propyl gallate, but mean body weights of dosed rats and mice of each sex were lower than those of the controls. At 104 weeks, mean body weights of low- and high-dose rats were 4% and 8% lower than those of the controls for males and 11% and 19% lower than those of the controls for females. Similarly, mean body weights of low- and high-dose mice were 5% and 8% lower than those of the controls for males and 11% (both dose groups) lower than those of the controls for females.

Thyroid follicular-cell adenomas or carcinomas (combined) occurred in male rats with a statistically significant ($P<0.05$) positive trend, but the incidences in the dosed groups were not statistically significant in direct comparisons with the control groups. Moreover, the incidence of high-dose male rats with follicular-cell tumors

(3/50, 6%) was not statistically different from the historical control rate (14/584, 2.4%) for the laboratory that conducted this bioassay.

Rare tumors (an astrocytoma or a glioma) were found in the brains of two low-dose female rats. The incidence of *all brain tumors in the Bioassay Program is only 0.86%.* The absence of this tumor in the high-dose female rat group reduces the likelihood that this tumor is related to propyl gallate administration.

Increased incidences of hepatic cytoplasmic vacuolization and suppurative inflammation of the prostate were observed in dosed male rats. These findings were considered to be related to administration of propyl gallate.

Tumors (mostly benign) of the preputial gland, islet-cell tumors of the pancreas, and pheochromocytomas of the adrenal gland were observed with significantly ($P<0.05$) higher incidences in the low-dose male rats, but there was little evidence of an effect in the high-dose group. The *incidences of male rats with tumors of the preputial gland* were 1/50 (2%) for controls, 8/50 (16%) for the low-dose, and 0/50 (0%) for the high-dose group. Islet-cell tumors of the pancreas occurred in 2/50 (4%) control males, 9/50 (18%) low-dose males, and 4/50 (8%) for high-dose males. Pheochromocytomas of the adrenal gland were observed in 4/50 (8%) control males, 13/48 (25%) low-dose males, and 8/50 (16%) high-dose males.

Negative trends ($P<0.05$) were observed for leukemia in male rats (16/50, 7/50, 6/50) and for fibroadenomas of the mammary gland in female rats (11/50, 2/50, 5/50).

In male mice, malignant lymphoma was observed with a significantly ($P \leq 0.014$) positive trend (control, 1/50, 2%; low-dose, 3/49, 6%; high-dose, 8/50, 16%), and the incidence in the high-dose group was significantly ($P \leq 0.028$) higher than that observed in the concurrent controls. However, the high-dose incidence was not statistically different from the historical rate (60/640, 9.4%) for the laboratory that conducted this bioassay.

Adenomas of the liver in female mice occurred with a statistically significant ($P \leq 0.022$) positive trend, and the incidence in the high-dose group was significantly ($P \leq 0.039$) higher than that of the controls (0/50, 0%; 2/50, 4%; 5/49, 10%). The incidences of hepatocellular adenomas or carcinomas (combined) were similar in control and dosed groups (3/50, 6%; 3/50, 6%; 5/49, 10%).

Negative trends ($P<0.05$) were obtained for fibromas of the skin or subcutaneous tissue in male mice (5/50, 1/49, 0/50).

Under the conditions of this bioassay, propyl gallate was not considered carcinogenic for F344/N rats, although there was evidence of an increased proportion of low-dose male rats with preputial gland tumors, islet-cell tumors of the pancreas, and pheochromocytomas of the adrenal glands; rare tumors of the brain occurred in two low-dose females. Propyl gallate was not considered to be carcinogenic for $B6C3F_1$ mice of either sex, although the increased incidence of malignant lymphoma in male mice may have been related to the dietary administration of propyl gallate.

Synonyms: 2,4,5 trihydroxybenzoic acid propyl ester; gallic acid propyl ester; Progallin P; Tennox PG

Report Date: December 1982

TR-241 Proceedings of a Working Seminar on the Optimal Use of Facilities for Carcinogenicity/Toxicity Testing

Note to the Reader: The proceedings of this working seminar held in May, 1980, were never published and are not available.

TR-242 Carcinogenesis Bioassay of Diallyl Phthalate (CAS No. 131-17-9) in $B6C3F_1$ Mice (Gavage Study)

Diallyl phthalate is a widely used crosslinking agent for unsaturated polyesters. Diallyl phthalate or diallyl phthalate polyester blends are used primarily as plasticizers and carriers for adding catalysts and pigments to polyesters and in molding, electrical parts, laminating compounds, and impregnation of metal castings. Rubber compounds, epoxy formulations, and polyurethane foams may also contain diallyl phthalate. Annual production of diallyl phthalate in the United States exceeds 5,000 pounds; precise figures are not available.

A carcinogenesis bioassay of diallyl phthalate (99% pure) was conducted by administering 0 (vehicle control), 150, or 300 mg/kg diallyl phthalate in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 male and 50 female $B6C3F_1$ mice. Survival rates and mean body weights of dosed mice were not different from those of the controls, and pathological lesions unrelated to proliferative changes were not observed. Therefore, a maximally tolerated dose for the purposes of carcinogenicity testing may not have been achieved.

The incidences of lymphoma and either lymphoma or leukemia in dosed male mice were no significantly greater than those in the controls according to pairwise comparisons ($P = 0.051$ to $P = 0.096$), but the trend tests were statistically significant by either life table or incidental tumor analysis ($P = 0.031$ to $P = 0.045$). The incidence of lymphomas in the high-dose male mice was 12/50 (24%) in comparison with 6/50 (12%) in the controls. Recent historical incidences at the performing laboratory and in the NTP Bioassay Program were 18/120 (15%) and 71/661 (11%), respectively. Since the incidence of high-dose male mice with leukemia was not significantly greater than that of concurrent or historical controls at the performing laboratory by pairwise comparisons, this marginal increase was considered only to be equivocally related to diallyl phthalate administration.

Increased incidences of squamous cell papillomas, hyperplasia, and inflammatory lesions of the forestomach were observed in diallyl phthalate-dosed mice of both sexes in a dose-related manner. Papillomas of the forestomach were observed in 0%, 2%, and 4% of the *control, low-dose, and high-dose mice of both sexes.* The recent historical incidence of this tumor in gavage control mice from both the performing laboratory and other laboratories within the Bioassay Program was less than 1%. Forestomach hyperplasia was diagnosed in 0%, 15%,

and 18%, and in 8%, 2%, and 29% of the control, low-dose, and high-dose male and female mice, respectively; chronic inflammation of the forestomach was diagnosed in 0%, 9%, and 16% and in 4%, 2%, and 18% of the control, low-dose, and high-dose male and female mice, respectively. Because of the numerical elevation of the forestomach papillomas in the high-dose mice of both sexes, the concomitant observation of dose related forestomach hyperplasia, and the rarity of this tumor in corn oil (gavage) control $B6C3F_1$ mice, the development of squamous cell papillomas of the forestomach may have been related to diallyl phthalate administration.

Under the conditions of this bioassay, the development of chronic inflammation and hyperplasia of the forestomach in both male and female $B6C3F_1$ mice was considered to be related to the administration of diallyl phthalate. The development of squamous cell papillomas of the forestomach may also have been related to chemical administration, but the available data are insufficient to indicate a clear cause and effect relationship. An increase in the incidence of male mice with lymphomas was observed, but this increase was considered only to be equivocally related to diallyl phthalate administration. The results of this bioassay, therefore, do not indicate that diallyl phthalate is carcinogenic in $B6C3F_1$ mice, although a maximum tolerated dose may not have been achieved. A carcinogenicity study by the National Toxicology Program of diallyl phthalate in male and female Fisher 344/N rats, employing daily gavage doses of 0 (vehicle control), 50, or 100 mg/kg body weight is currently being evaluated.

Report Date: April 1983

Note: Diallyl Phthalate was subsequently tested in F344 rats by gavage (See TR-284, reported 1985).

TR-243 Carcinogenesis Studies of Trichloroethylene (Without Epichlorohydrin) (CAS No. 79-01-6) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Trichloroethylene (TCE) is an industrial solvent used for vapor degreasing and cold cleaning of fabricated metal parts. TCE has also been used as a carrier solvent for the active ingredients of insecticides and fungicides, as a solvent for waxes, fats, resins, and oils, as an anesthetic for medical and dental use, and as an extractant for spice oleoresins and for caffeine from coffee. Trichloroethylene may be found in printing inks, varnishes, adhesives, paints, lacquers, spot removers, rug cleaners, disinfectants, and cosmetic cleansing fluids. TCE may also be used as a chain terminator in polyvinyl chloride production and as an intermediate in the production of pentachloroethane. Trichloroethylene is no longer used with food, drugs, or cosmetics.

Carcinogenesis studies of epichlorohydrin-free trichloroethylene were conducted by administering the test chemical in corn oil by gavage to groups of 50 male and 50 female F344/N rats and $B6C3F_1$ mice. Dosage levels were 500 and 1,000 mg/kg for rats and 1,000 mg/kg for mice. Trichloroethylene was administered five times per week for 103 weeks, and surviving animals were killed between weeks 103 and 107. Groups of 50 rats and 50 mice of each sex received corn oil by gavage on the same schedule and served as vehicle controls. Groups of 50 male and 50 female rats were used as untreated controls.

The dosage levels selected for the 2-year study were based on the results of the 13-week studies. Groups of 10 male and 10 female rats received TCE by gavage at doses of 125 to 2,000 mg/kg (males) and 62.5 to 1,000 mg/kg (females) for 13 weeks. Groups of 10 male and 10 female mice received gavage doses of 375 to 6,000 mg/kg of TCE for 13 weeks. Survival, body weight gains, and previous experience with TCE were used to select doses for the 2-year study. All rats survived the 13-week study, but males receiving 2,000 mg/kg exhibited a 24% difference in final body weight. At the 1,000 mg/kg dose, final body weights for males (-3%) and for females (-2%) were similar to those of controls. The doses selected for the 2-year study in rats were 500 and 1,000 mg/kg for both sexes. The initial doses used in the earlier bioassay in Osborne-Mendel rats were 549 and 1,097 mg/kg for both sexes. A total of 8/10 male mice and 10/10 female mice receiving doses of TCE as high as 1,500 mg/kg survived the 13-week experimental period. The single dosage level selected for the 2-year study in mice was 1,000 mg/kg for both sexes. This dose was less than the high dose used in the earlier bioassay in $B6C3F_1$ mice (2,339 mg/kg for males and 1,739 for females) and was similar to the previous low doses (1,169 mg/kg for males and 869 for females).

In the 2-year study, the survival of both low and high dose male rats and dosed male mice was less ($P \leq 0.005$) than that of the vehicle controls. Mean body weights of dosed rats of each sex were lower than those of the vehicle controls, and after week 65, the decrements in body weight gains were dose related. The mean body weight of dosed male mice was lower than that of the vehicle controls throughout the study, while those of the dosed and vehicle control female mice were comparable.

Cytomegaly (toxic nephrosis) of the kidney was observed in 96/98 male and in 97/97 female rats given TCE, with none being found in male or female vehicle control rats. This lesion was more severe in males, particularly in the high dose group. Cytomegaly was observed in 45/50 male mice and in 48/49 female mice administered TCE, and in none of the vehicle controls. Renal tubular cell adenocarcinomas were found in the three high dose male rats; these neoplasms were observed in those male rats killed at the end of the study (0/33, 0/20, and 3/16, 19%). The incidence in the high dose male rats at the end of the study was greater ($P<0.05$) than that in the controls. Renal tubular cell adenocarcinomas are considered uncommon occurrences in F344/N rats, with 3/748

(0.4%) being observed in historical vehicle gavage controls. Additional renal tumors in dosed male rats included one transitional cell carcinoma of the renal pelvis and two tubular cell adenomas in low dose animals and one carcinoma of the renal pelvis in a high dose animal. No renal neoplasms were found in vehicle control rats; one untreated control male rat had a transitional cell papilloma of the renal pelvis. In female rats, one tubular cell adenocarcinoma was found in the high dose group.

An increased incidence ($P<0.05$, life table) of peritoneal mesotheliomas was detected in low dose male rats (control, 1/50; low dose, 5/50; high dose, 1/49). Mesotheliomas have been diagnosed in 16/752 (2.1%) historical vehicle control male F344/N rats, and the increased incidence in the present study may have been related to the administration of TCE.

The results in male F344/N rats were considered equivocal for detecting a carcinogenic response because both groups receiving TCE showed significantly reduced survival compared to vehicle controls (35/50, 70%, 20/50, 40%; 16/50, 32%) and because 20% of the animals in the high dose group were killed accidently by gavage error.

Negative trends were observed for chromophobe adenomas of the pituitary gland and for endometrial stomal polyps in female rats. These decreases were not considered to be related to the administration of TCE.

The administration of TCE to mice caused increased incidences of hepatocellular carcinoma in males (control, 8/48; dosed, 31/50; $P<0.001$) and in females (control, 2/48; dosed, 13/49; $P<0.005$). Hepatocellular carcinomas metastasized to the lungs in five dosed male mice and one control male mouse, and none were observed in females. The incidence of hepatocellular adenomas was increased in male mice (control, 7/48; dosed 14/50) and in female mice (control, 4/48; dosed, 16/49; $P<0.05$).

Under the conditions of these studies, epichlorohydrin-free trichloroethylene caused renal tubular-cell neoplasms in male F344/N rats, produced toxic nephrosis in both sexes, and shortened the survival time of males. This experiment in male F344/N rats was considered to be inadequate to evaluate the presence or absence of a carcinogenic response to trichloroethylene. For female F344/N rats receiving trichloroethylene, containing no epichlorohydrin, there was no evidence of carcinogenicity. Trichloroethylene (without epichlorohydrin) was carcinogenic for $B6C3F_1$ mice, causing increased incidences of hepatocellular carcinomas in males and females and of hepatocellular adenomas in females.

Synonym: TCE

Report Date: May 1990

Note: Trichloroethylene was previously tested in Osborne-Mendel rats and $B6C3F_1$ mice by gavage (See TR-2, reported 1977) and also in four rat strains (ACI, August, Marshall, and Osborne-Mendel) by gavage (See TR-273, reported 1988).

TR-244 Toxicology and Carcinogenesis Studies of a Polybrominated Biphenyl Mixture (Firemaster FF-1) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Firemaster FF-1, a flame retardant composed of polybrominated biphenyls (PBB), was responsible for widespread environmental contamination and animal losses in Michigan starting in 1973. This study was undertaken to characterize the long-term toxic and carcinogenic potential of this PBB mixture in rats and mice of each sex. Fisher 344/N rats and $B6C3F_1$ mice were given 125 oral doses of PBB over a 6-month period – 0, 0.1, 0.3, 1.0, 3.0, or 10.0 mg/kg body weight/day (5 days/week).

A dose-related decrease in body weight gain was observed in both male and female rats and male mice, although there was no significant difference in food consumption. At the end of the 6-month exposure, there was a dose-dependent decrease in thymus weights in rats. The liver appeared to be the primary target organ. Dose-related hepatotoxic effects were characterized by a marked increase in liver weight, with accentuation of hepatic lobular markings. Microscopically, there was moderate to marked hepatocellular swelling, disorganization and single cell necrosis of hepatocytes, fatty infiltration, and bile duct proliferation. At the 6-month observation, atypical hepatocellular foci were observed at a low incidence in dosed rats and mice. Hepatic porphyrin levels were markedly increased in both rats and mice, excessively in females. Levels of porphyrin tended to decrease gradually, primarily in mice, following cessation of exposure. The significant decreases in serum thyroxine (T_4) and triiodothyronine (T_3) in rats suggest that PBB may interfere with thyroid hormone secretion.

Total serum protein was decreased in dose-related fashion in female rats primarily due to dose-related decreases in albumin. There was a significant increase in the serum levels of gamma glutamyl transpeptidase (GGTP) in female rats given 10.0 mg/kg of PBB. There was a dose-related decrease in serum glucose in female rats, a dose-related decrease in the serum triglyceride level in dosed male rats, except at the lowest dose (0.1 mg/kg), and a dose-related increase in the serum levels of cholesterol in both male and female rats.

Serum levels of GGTP were increased only in female mice given 10.0 mg/kg of PBB. There was a 5- to 6-fold increase in the activity of serum glutamic pyruvic transaminase (SGPT) in male and female mice in the 10.0 mg/kg groups. Serum enzyme activity of alkaline phosphate (AP) was also increased in mice given the highest dose of PBB. There was a significant dose-related increase in the serum levels of cholesterol in female mice, and the highest dose group was significantly greater than the control female mice. Serum glucose was significantly decreased in female mice administered 10.0 mg/kg of PBB.

To determine the carcinogenic potential of PBB, rats and mice dosed for 6 months were observed without exposure to PBB for an additional 23 or 24 months, respectively (lifetime observation). The dosing (0.3 mg/

kg or higher dose levels) shortened the survival time in male rats, whereas no such effect was observed in dosed females. There was also evidence of shortened survival time in the 10.0 mg/kg PBB-dosed mice. A significantly higher incidence of atypical hepatocellular foci, neoplastic nodules, hepatocellular carcinomas, and cholangiocarcinomas was observed in dosed rats. The incidence of hepatocellular carcinoma was increased in both male and female mice (highest dose level) compared with control male and female mice. The incidence of hepatic neoplasms appeared to be dose dependent in rats and mice. Liver tumors were observed primarily in those groups of animals to which PBB was given in doses sufficient to induce readily observable hepatic toxicity.

Under the conditions of these studies, polybrominated biphenyl mixture (Firemaster FF-1) was carcinogenic for Fisher 344/N rats and $B6C3F_1$ mice of each sex, inducing neoplastic nodules, hepatocellular carcinomas, and cholangiocarcinomas in rats and hepatocellular carcinomas in mice. Other toxicities included porphyrogenic effects and hepatotoxicity.

Synonym: Firemaster FF-1

Report Date: June 1983

TR-245 Carcinogenesis Bioassay of Melamine (CAS No. 108-78-1) in F344/N Rats and B6C3F1 Mice (Feed Study)

A carcinogenesis bioassay of melamine (>95% pure), a chemical intermediate in the manufacture of amino resins and plastics, was conducted by feeding diets containing 2,250 or 4,500 ppm melamine to groups of 50 male F344/N rats and 50 $B6C3F_1$ mice of each sex for 103 weeks. Groups of 49 male rats, 50 female rats, 49 male mice, and 50 female mice served as controls.

Mean body weights of dosed rats of each sex were lower than those of the controls after week 20. Survival of high-dose male rats was significantly lower ($P \leq 0.05$) than that of the controls. Survival of all other dosed rat groups was comparable with that of the respective controls.

Transitional-cell carcinomas in the urinary bladder of male rats occurred with a statistically significant positive trend($P \leq 0.002$; controls, 0/45; low-dose, 0/50; high-dose, 8/49, 16%) and the incidence in the high-dose group was significantly higher($P \leq 0.016$) than that in the controls. A transitional-cell papilloma was observed in the urinary bladder of an additional high-dose male rat. These tumors were not observed in statistically significant proportions in female rats. Seven of the eight high-dose male rats with the transitional-cell carcinomas also had bladder stones. An association ($P \leq 0.001$) was found between bladder stones and bladder tumors in male rats.

Chronic inflammation, distinguishable from the nephropathy observed in aging F344/N rats, was significantly increased ($P \leq 0.01$) in the kidney of dosed female rats (controls, 4/50, 8%; low-dose, 17/50, 34%; high-dose, 41/50, 82%) and is attributed to the administration of melamine.

The mean body weight of high-dose male mice was lower than that of controls after week 50 of the study. The mean body weights of dosed and control female mice were comparable throughout the study. Survival of high-dose male mice was significantly less ($P<0.02$) than that of the controls. Survival of all other dosed groups was similar to that of the respective controls.

Acute and chronic inflammation and epithelial hyperplasia of the urinary bladder were found in increased incidence in dosed male mice. The incidence of bladder stones in dosed male mice was increased relative to controls (control, 2/45, 4%; low dose, 40/47, 85%; high-dose, 41/45, 93%); however, there was no evidence of bladder tumor development in this species. Also, four high-dose female mice had bladder stones without any tumors.

Under the conditions of this bioassay, melamine was carcinogenic for male F344/N rats, causing transitional-cell carcinomas in the urinary bladder. With one exception, urinary bladder stones were observed in male rats that had transitional-cell carcinomas. Melamine was not carcinogenic for female F344/N rats or for $B6C3F_1$ mice of either sex.

Synonyms: 2,4,6-triamino-*s*-triazine; cyanurotriamide

Report Date: March 1983

TR-246 Lifetime Carcinogenesis Studies of Chrysotile Asbestos (CAS No. 12001-29-5) in Syrian Golden Hamsters (Feed Studies)

Carcinogenesis studies of short range (SR), intermediate range (IR), or intermediate range chrysotile asbestos in combination with the intestinal carcinogen 1,2-dimethylhydrazine dihydrochloride (DMH) were conducted with male and female Syrian golden hamsters. Both forms of chrysotile asbestos were administered at the concentration of 1% in pelleted diet for the entire lifetime of the hamsters starting with mothers of the test animals. Group sizes varied from 125 to 253. Starting at 6 weeks of age, male and female hamsters in the intermediate range chrysotile/DMH study were given oral doses of DMH (4 mg/kg) every other week for a total of 5 doses. There was no adverse effect on body weight gain or survival by either form of asbestos or by asbestos in combination with DMH.

A significant increase ($P<0.05$) in adrenal cortical adenomas was observed in male hamsters exposed to SR and IR chrysotile asbestos and in females treated with IR chrysotile asbestos when compared to the pooled control groups (males: pooled controls, 25/466, 5%; SR chrysotile, 26/299, 11%; IR chrysotile, 24/244, 10%; females: pooled controls, 15/468, 3%; IR chrysotile, 18/234, 8%). However, statistical significance was lost when these dosed groups were compared with concur-

rent control groups (males: SR control, 7/115, 6%; IR control, 7/115, 6%; females: SR control, 4/112, 4%; IR control, 6/118, 5%).

The results of the combination study (IR chrysotile plus DMH) did not yield a significant increase in tumors above the background level observed in the DMH group alone or in the untreated control group. The DMH failed to yield a background level of intestinal tumors high enough to provide a valid test of the cocarcinogenic potential of chrysotile asbestos. For this reason, the cocarcinogenic potential of orally administered asbestos should be considered untested.

Under the conditions of these studies, neither short range chrysotile nor intermediate range chrysotile asbestos was carcinogenic when ingested at 1% levels in the diet by male and female Syrian golden hamsters. While there were increases in the rates of adrenal cortical adenomas in male and female hamsters exposed to intermediate range chrysotile asbestos compared to the pooled groups, these incidence rates were not different when compared with the concurrent control groups. Additionally, the biologic importance of adrenal tumors in the absence of target organ (gastrointestinal tract) neoplasia is questionable. The cocarcinogenesis studies using IR chrysotile asbestos and 1,2-dimethylhydrazine dihydrochloride were considered inadequate because there was no increase in intestinal neoplasia in the DMH group.

Report Date: July 1990

Note: Chrysotile Asbestos was also tested in F344 rats administered in feed (See TR-295, reported 1985).

TR-247 Carcinogenesis Bioassay of L-Ascorbic Acid (Vitamin C) (CAS No. 50-81-7) in F344/N Rats and B6C3F$_1$ Mice (Feed Study)

L-Ascorbic acid is essential for many physiologic functions in animals and humans, mostly biochemical reactions involving oxidation.

L-Ascorbic acid is approved for use as a dietary supplement and chemical preservative by the U.S. Food and Drug Administration and is on the FDA's list of substances generally recognized as safe.

L-Ascorbic acid may be used in soft drinks as an antioxidant for flavor ingredients, in meat and meat-containing products, for curing and pickling, in flour to improve baking quality, in beer as a stabilizer, in fats and oils as an antioxidant, and in a wide variety of foods for vitamin C enrichment. L-Ascorbic acid may also find use in stain removers, hair waving preparations; plastics manufacture, photography, and water treatment.

A carcinogenesis bioassay of L-ascorbic acid (>97% pure) was conducted by administering diets containing 25,000 or 50,000 ppm L-ascorbic acid to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex for 103 weeks. Controls consisted of 50 untreated rats and untreated mice of each sex. Fifty-thousand ppm is the highest dose recommended for chronic studies.

Survival of dosed and control female rats and of dosed and control female mice were comparable. Survival of high-dose male rats was slightly greater than that of the controls ($P = 0.087$). Survival of high-dose male mice was significantly greater ($P = 0.009$) than that of the controls. Throughout most of the study, mean body weights of dosed female rats and dosed female mice were lower than those of the controls. Final body weights were comparable among groups, except for the high-dose female rats (<13%); marginal differences (<8%) were observed for low-dose female rats and for dosed female mice (8%-11%). Food consumption was equivalent among groups.

Most observational differences were confined to the female rat. The incidence of low-dose female rats with undifferentiated (mononuclear-cell) leukemias (control, 6/50, 12%; low-dose, 17/50, 34%; high-dose, 12/50, 24%) was significantly higher ($P<0.02$) than that in controls. These tumors were not considered to be related to administration of L-ascorbic acid because they did not occur in the female high-dose group at incidences significantly greater ($P>0.07$) than those in the controls, the trend test was not significant ($P\geq0.07$), and no increases were observed for male rats.

Under the conditions of this bioassay, L-ascorbic acid was not carcinogenic for male and female F344/N rats or male and female B6C3F$_1$ mice.

Synonym: vitamin C

Report Date: March 1983

TR-248 Carcinogenesis Studies of 4,4'-Methylenedianiline Dihydrochloride (CAS No. 13552-44-8) in F344/N Rats and B6C3F$_1$ Mice (Drinking Water Studies)

4,4'-Methylenedianiline is used primarily as a chemical intermediate in the closed system production of isocyanates and polyisocyanates. These chemicals are used extensively in the manufacture of rigid polyurethane foams for thermal insulation and in the production of semiflexible polyurethane foams for automobile safety cushioning. The saturated isocyante of 4,4'-methylenedianiline [4,4'-methylene-bis(cyclohexylisocyanate)] is an intermediate in the production of light-stable, high-performance polyurethane coatings. 4,4'-Methylenedianiline is also a curing agent for epoxy resins and urethane elastomers, a dye intermediate, and a corrosion inhibitor.

Carcinogenesis studies of 4,4'-methylenedianiline dihydrochloride (98.6% pure) were conducted by administering this chemical in the drinking water of F344/N rats and B6C3F$_1$ mice. Groups of 50 rats and 50 mice of each sex received drinking water containing 150 or 300 ppm 4,4'-methylenedianiline dihydrochloride (dosage expressed as the free base) for 103 weeks. Groups of 50

rats and 50 mice of each sex, given drinking water adjusted with 0.1N HCl to the pH (3.7) of the 300-ppm formulation, served as controls.

Survival was comparable among groups except for male mice receiving the high dose of 4,4'-methylenedianiline dihydrochloride; survival in that group was lower ($P = 0.006$) than that in controls. Mean body weight was reduced in high dose female rats and in high dose male and female mice. Water consumption was reduced in a dose-related manner in both sexes of rats. No compound-related clinical effects were observed.

Compound-related nonneoplastic lesions of the thyroid in female rats included follicular cysts and hyperplasia. The incidence of thyroid follicular cell hyperplasia was elevated in high dose male and female mice. The incidences of thyroid neoplasms in the high dose groups were elevated compared with those of the control groups for both sexes of both species. Thyroid follicular cell carcinoma was increased in male rats (controls, 0/49; low dose, 0/47; high dose, 7/48, 15%: $P \leq 0.012$). Follicular cell adenoma was increased in high dose female rats (0/47; 2/47, 4%; 17/48, 35%: $P < 0.001$), in high dose male mice (0/47; 3/49, 6%; 16/49, 33%: $P < 0.001$), and in high dose female mice (0/50; 1/47, 2%; 13/50, 26%: $P < 0.001$) as compared with controls. In female rats, thyroid C-cell adenoma was also elevated in a dose-related manner (0/47; 3/47, 6%; 6/48, 13%, $P \leq 0.029$).

Dose-related increases in nonneoplastic lesions were observed for male rats (nonspecific liver dilatation) and for male and female rats (fatty metamorphosis and focal cellular change). Liver degeneration was present in 80% of the low dose and 60% of the high dose male mice but was not found in the controls. Neoplastic nodules of the liver were observed at greater incidences ($P \leq 0.002$) for low and high dose male rats as compared with controls (control, 1/50, 2%; low dose, 12/50, 24%, $P \leq 0.002$; high dose 25/50, 50%, $P < 0.001$). Hepatocellular adenoma was increased in a dose-related manner in dosed female mice (3/50, 6%; 9/50, 18%; 12/50, 24%, $P < 0.011$). Hepatocellular carcinoma was observed in greater incidence in dosed male mice (10/49, 20%; 33/50, 66%, $P < 0.001$; 29/50, 58%, $P < 0.001$) and in high dose female mice (1/50, 2%; 6/50, 12%; 11/50, 22%, $P = 0.002$).

Male rats had a dose related increase in kidney mineralization. Nephropathy was increased in dosed mice of both sexes; renal papillary mineralization was greater in high dose male mice and female mice than in the controls.

Other tumors that were elevated in dosed animals included adrenal pheochromocytomas in male mice (control, 2/48, 4%; low dose, 12/49, 24%, $P \leq 0.006$; high dose, 14/49, 29%; $P \leq 0.001$), alveolar/bronchiolar adenoma in female mice (1/50, 2%; 2/50, 4%; 6/49, 12%, $P \leq 0.05$) and malignant lymphomas in female mice (13/50,26%; 28/50, 56%, $P = 0.002$; 29/50, 58%; $P = 0.001$).

Uncommon tumors were observed in dosed animals at low incidences but may be important because the historical control incidences are very low; bile duct adenoma in 1/50 high dose male rats (historical control 3/3,663), transitional-cell papillomas of the urinary bladder in female rats (historical control, 3/3,664, 0.08%; low dose, 2/50, 4%; high dose, 1/50, 2%) and granulosa cell tumors of the ovary in female rats (historical control, 11/3,642, 0.3%; low dose, 3/50, 6%; high dose, 2/50, 4%).

Decreases in tumor incidences were observed for leukemia in male rats (control, 12/50, 24%; low dose, 6/50, 12%; high dose, 5/50, 10%, $P = 0.048$) and alveolar or bronchiolar adenomas (combined) in male mice (12/49, 24%; 9/49, 18%; 3/49, 6%, $P \leq 0.011$).

Under the conditions of these studies, 4,4'-methylenedianiline dihydrochloride was carcinogenic for F344/N rats and $B6C3F_1$ mice of each sex, causing significantly increased incidences of thyroid follicular cell carcinomas in male rats, thyroid follicular cell adenomas in female rats and in mice of each sex, C-cell adenomas of the thyroid gland in female rats, neoplastic nodules in the liver of male rats, hepatocellular carcinomas in mice of each sex, adenomas of the liver and malignant lymphomas in female mice, and adrenal pheochromocytomas in male mice.

Report Date: June 1983

TR-249 Lifetime Carcinogenesis Studies of Amosite Asbestos (CAS No. 12172-73-5) in Syrian Golden Hamsters (Feed Studies)

Carcinogenesis studies of amosite asbestos were conducted by administering diets containing 1% of the asbestos in pellets from the conception of the mothers through the lifetime of male and female Syrian golden hamsters. Control groups consisted of 127 male and 126 female hamsters and the amosite asbestos groups consisted of 252 male and 254 female hamsters.

No adverse effect on body weight gain or survival was observed from treatment with amosite asbestos. Neither of the amosite asbestos groups showed increased neoplasia in any organ or tissue compared to the control groups.

Under the conditions of these studies, the ingestion of amosite asbestos at a level of 1% in the diet for their lifetime was not toxic and did not cause a carcinogenic response in male and female Syrian golden hamsters.

Report Date: November 1983

Note: Amosite Asbestos was subsequently tested in F344 rats administered in feed (See TR-279, reported 1990).

TR-250 Toxicology and Carcinogenesis Studies of Benzyl Acetate (CAS No. 140-11-4) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Benzyl acetate, a water-white liquid with a pear-like odor, is a natural constituent of several essential oils and flower absolutes extracted from jasmine, hyacinth, gar-

denia, tuberose, ylang-ylang, cananga, and neroli. Commercial benzyl acetate, a liquid prepared synthetically from benzyl chloride, acetic acid, and triethylamine is used primarily as a component of perfumes for soaps and as a flavoring ingredient. This compound is practically insoluble in water but is miscible in alcohol and ether and soluble in benzene and chloroform.

Toxicology and carcinogenesis studies of benzyl acetate (>99% pure) were conducted by administering benzyl acetate in corn oil gavage to groups of 50 male and 50 female F344/N rats at doses of 0, 250, or 500 mg/kg body weight and to groups of 50 male and 50 female $B6C3F_1$ mice at doses of 0, 500, or 1,000 mg/kg once daily five days per week for 103 weeks. Dose selection for the 2-year study was based on mean body weight gain depression and decreased survival observed at higher doses in 13 week studies.

The absence of any observable adverse effect of benzyl acetate on the survival or mean body weight gains of the rats or mice in the 2-year studies suggests that both the rats and the mice of each sex could have tolerated higher doses. An infection in the genital tract was probably responsible for the deaths of 26/35 control, 14/32 low-dose, and 8/20 high-dose female mice before the end of the study.

Acinar-cell adenomas in the pancreas of male rats occurred with a positive trend ($P<0.01$), and the incidence in the high-dose group (37/49, 76%) was significantly ($P<0.01$) higher than in the vehicle controls (22/50, 40%). The incidence of these tumors in the low-dose group (27/50, 54%) was comparable to that in the gavage controls. Acinar-cell hyperplasia of the pancreas was observed in 37/50 control, 34/50 low-dose, and 36/49 high-dose male rats. No acinar-cell hyperplasia or adenoma of the pancreas was observed in female rats.

The incidence of retinopathy and cataracts in the high-dose male rats was increased compared with the controls (retinopathy: 1/50; 0/50; 20/50; cataracts: 0/50; 0/50; 13/50). Low-dose female rats had an increased incidence of retinopathy (18/50). Retinopathy and cataracts in rats have been associated with proximity to fluorescent light in this and previous studies.

Preputial gland neoplasms occurred with a positive trend ($P<0.05$) in male rats (cystadenocarcinoma: 0/50; 0/50; 3/50; all adenocarcinoma: 0/50; 1/50; 4/50; adenocarcinoma or carcinoma combined: 1/50; 1/50; 6/50). However, the incidence of all preputial gland tumors was not significantly elevated (2/50; 1/50; 6/50). For female rats the incidence of clitoral gland neoplasms was marginally increased (2/50; 0/50; 5/50).

Hepatocellular adenomas occurred in mice of each sex with statistically positive trends (males: 0/50; 5/49; 13/50; females: 0/50; 0/50; 6/50), and the incidences in the high-dose groups were greater than those in the controls (males: $P<0.001$; females: $P<0.05$). Hepatocellular carcinomas were marginally elevated in dosed male and high-dose female mice (males: 10/50; 14/49; 12/50; females: 1/50; 0/50; 4/50).

Squamous cell papillomas or carcinomas of the forestomach (uncommon neoplasms) occurred with a positive trend ($P<0.05$) in male mice (4/49; 4/48; 11/49). The incidence of these tumors was also marginally ($P=0.054$) increased in the high-dose female mice (0/50; 0/50; 4/48). The incidences of these tumors in both the high-dose male and the high-dose female mice were considerably higher than the historical corn oil gavage control rates at this laboratory (males, 2/296, 0.7%; females, 2/297, 0.7%) and throughout the program (males, 14/1,070, 1.3%; females, 3/1,073, 0.3%). Forestomach hyperplasia occurred at increased incidences in dosed mice of either sex (males: 1/49, 7/48, 22/49; females: 1/50, 6/50, 17/48). The neoplasms and hyperplasia of the forestomach were probably related to administration of benzyl acetate.

In a separate metabolism study, benzyl acetate was absorbed from the gastrointestinal tract of rats and mice, with approximately 90% of the administered dose recovered as various metabolites in the urine within 24 hr. The primary metabolite was hippuric acid, with minor amounts of a mercapturic acid, and one or more unidentified metabolites. This capacity for absorption, metabolism, and disposition was unaffected by the amount or number of doses administered.

Benzyl acetate was not mutagenic in strains TA100, TA98, TA135, or TA137 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced Sprague-Dawley rat or Syrian hamster S9 when tested according to the preincubation protocol. Benzyl acetate did not induce sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. Benzyl acetate was mutagenic in the mouse lymphoma L5178Y/$TK^{+/-}$ assay in the presence, but not in the absence, of Aroclor 1254-induced Fisher 344 rat liver S9.

An audit was conducted on the experimental data and the draft technical report for these 2-year studies on benzyl acetate. Based on the results of this audit additional pathology examinations were conducted on all target organs in male rats and male and female mice. The Technical Report reflects these final pathology evaluations. The overall conclusions regarding the toxicology and carcinogenicity of benzyl acetate did not change as a result of this evaluation.

Under the conditions of these gavage studies, benzyl acetate increased the incidence of acinar-cell adenomas of the exocrine pancreas in male F344/N rats; the gavage vehicle may have been a contributing factor. There was *no evidence of carcinogenicity* for female F344/N rats. For male and female $B6C3F_1$ mice there was *some evidence of carcinogenicity* in that benzyl acetate caused increased incidences of hepatocellular adenomas and squamous cell neoplasms of the forestomach.

Synonyms: alpha-acetoxytoluene; benzyl ethanoate; acetic acid, benzyl ester

Report Date: August 1986

TR-251 Toxicology and Carcinogenesis Studies of Commercial Grade 2,4 (80%)- and 2,6 (20%)- Toluene Diisocyanate (CAS No. 26471-62-5) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Toluene diisocyanate (TDI) is commercially produced as an approximate 80:20 mixture of the 2,4- and 2,6-isomers. In 1980, 580,000 pounds of this chemical were produced in the United States, primarily for use in the manufacture of flexible polyurethane foams. These foam elastomers are found in furniture and automobile cushions, carpet underlays, pillow filling, mattresses, insulation, shoes, purses, and toys. TDI is also used to produce polyurethane coatings for lacquers and wood finishes.

Groups of 50 female F344/N rats and 50 B6C3F$_1$ mice were administered commercial grade toluene diisocyanate (80% 2,4- and 20% 2,6-) in corn oil by gavage at doses of 60 or 120 mg/kg body weight, 5 days per week for 105 or 106 weeks. Groups of 50 male F344/N rats received 30 or 60 mg/kg and groups of 50 male B6C3F$_1$ mice received 120 or 240 mg/kg on the same schedule. Dosage analyses of toluene diisocyanate indicated that the chemical had reacted in the corn oil vehicle, resulting in actual gavage concentrations 77% to 90% of theoretical values. Groups of 50 rats and 50 mice of each sex received corn oil only and served as vehicle controls.

Survival in all groups of dosed rats in the 2-year studies were shorter ($P \leq 0.005$) than that of the controls; depressions of the mean body weight gain relative to controls were greater than 10% in all dosed rat groups throughout most of the study. A dose-dependent pattern of cumulative toxicity began at 70 weeks and culminated in excessive mortality, indicating the estimated tolerated dose had been exceeded for rats. Acute bronchopneumonia occurred at increased incidences in groups of dosed male and female rats (males: control, 2/50; low dose, 6/50; high dose, 14/50; females: 1/50, 10/50, 25/49).

Subcutaneous tissue fibromas or fibrosarcomas (combined) in male rats occurred with a positive trend ($P<0.01$; 3/50, 6/50, 12/50). The incidence in the high dose group was higher than that in the controls ($P \leq 0.01$). The same tumor comparisons were significant ($P<0.001$) in female rats by the life table analysis. Mammary gland fibroadenomas in female rats occurred with a positive trend ($P<0.001$), and the incidences in low and high dose groups were significantly higher than that in controls ($P \leq 0.01$).

Pancreatic acinar cell adenomas in male rats occurred with a positive trend ($P<0.05$; 1/47, 3/47, 7/49). The incidence in the high dose group was higher than that in the controls ($P<0.05$).

The incidences of pancreatic islet cell adenomas in female rats were higher by the incidental tumor test ($P \leq 0.01$) in low dose (6/49) and high dose (2/47) groups than in controls (0/50). An islet cell carcinoma was also observed in a low dose female rat. The incidences of female rats with neoplastic nodules in the liver occurred with a positive trend ($P<0.05$; 3/50, 8/50, 8/48), and the incidence in the high dose group was higher ($P<0.05$) than that in the controls.

Survival of high dose male mice in the 2-year study was significantly shorter than that of the controls ($P<0.001$). During the second year of the study, mean body weight gains of high dose male mice were less than those of the controls. Cytomegaly of kidney tubular epithelium was found in 45/48 (94%) low dose male mice and 41/50 (82%) high dose male mice but not in any of the controls.

Hemangiomas or hemangiosarcomas (combined) of the circulatory system in female mice occurred with a positive trend ($P \leq 0.01$; control, 0/50; low dose, 1/50; high dose, 5/50). The incidence in the high dose group was significantly higher than that in the controls ($P<0.05$).

Hepatocellular adenomas in female mice occurred with a positive trend ($P \leq 0.001$; 2/50, 3/50, 12/50), and the incidence in the high dose group was higher than that in the controls ($P<0.01$).

Toluene diisocyanate was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 in the presence (but not the absence) of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9; it was not mutagenic in strains TA 1535 or 1537.

An audit of the experimental data for these 2-year toxicological and carcinogenicity studies on commercial grade 2,4- and 2,6-toluene diisocyanate was conducted. There were no data discrepancies that influenced the final interpretations.

Under the conditions of these gavage studies, commercial grade toluene diisocyanate in corn oil was carcinogenic for F344/N rats, causing subcutaneous fibromas and fibrosarcomas (combined) in males and females, pancreatic acinar cell adenomas in males, and pancreatic islet cell adenomas, neoplastic nodules of the liver, and mammary gland fibroadenomas in females. Toluene diisocyanate was not carcinogenic for male B6C3F$_1$ mice. TDI was carcinogenic for female B6C3F$_1$ mice, causing hemangiomas or hemangiosarcomas (combined), as well as hepatocellular adenomas.

Synonym: TDI

Report Date: August 1986

TR-252 Carcinogenesis Studies of Food Grade Geranyl Acetate (71% Geranyl Acetate, 29% Citronellyl Acetate) (CAS No. 105-87-3) in F344/N Rats and B6C3F$_1$ Mice (Gavage Study)

Geranyl acetate is a colorless liquid prepared by fractional distillation of selected essential oils or by acetylation of geraniol. It is a natural constituent of more than 60 essential oils, including Ceylon citronella, palmarosa, lemon grass, petit grain, neroli bigarade, geranium, coriander, carrot, and sassafras.

Geranyl acetate is used primarily as a component of perfumes for creams and soaps and as a flavoring ingre-

dient. On the U.S. Food and Drug Administration's list of substances "generally recognized as safe", the Food Chemicals Codex (1972) specifies that geranyl acetate must contain at least 90% total esters.

Carcinogenesis studies of food-grade geranyl acetate (containing approximately 29% citronellyl acetate) were conducted by administering the test chemical in corn oil by gavage to groups of 50 male and 50 female F344/N rats at doses of 1,000 or 2,000 mg/kg body weight and to groups of 50 male and 50 female $B6C3F_1$ mice at doses of 500 or 1,000 mg/kg. Doses were administered five times per week for 103 weeks. Groups of 50 rats and 50 mice of each sex received corn oil by gavage on the same dosing schedule and served as vehicle controls.

The cumulative toxicity of geranyl acetate in the 2-year study was indicated by the significantly shorter survival of high dose male rats (control, 34/50; low dose, 29/50; high dose, 18/50) and of high dose male mice (control, 31/50; low dose, 32/50; high dose, 0/50) and of dosed female mice (38/50; 15/50; 0/50) when compared with controls. Throughout most of the 2-year study, mean body weights of high dose rats and mice of each sex were lower than those of the controls.

The occurrence of retinopathy or cataracts in the high dose male rats and low dose female rats as compared with the controls does not appear to be related to the administration of geranyl acetate but rather the proximity of the rats to fluorescent light. The incidence of retinopathy or cataracts (combined) was: males: control, 0/50, 0%; low dose, 1/50, 2%; high dose, 11/50, 22%; females: control, 1/50, 2%; low dose, 13/50, 26%; high dose, 2/50, 4%. Kidney tubular cell adenomas, an uncommon tumor type, were found in 2/50 (4%) low dose male rats. The historical incidence of male corn oil gavage control F344/N rats with kidney tumors is 1/250 (0.4%) at this laboratory and 4/998 (0.4%) in the program.

Squamous cell papillomas in the skin were increased marginally in low dose male rats (control, 0/50; low dose, 4/50, 8%; high dose, 1/50, 2%). In addition, one low dose male rat had a squamous cell carcinoma of the skin. The incidence of low dose male rats with either squamous cell papillomas or carcinomas was greater ($P<0.05$) in comparison with the controls. The historical incidence of squamous cell papillomas or carcinomas (combined) in gavage control male F344/N rats is 3.6% (9/250) at this laboratory and 2.5% (25/999) throughout the program. The incidence of all epidermal tumors was not significantly elevated in dosed male rats relative to controls (control, 3/50, 6%; low dose, 6/50, 12%; high dose, 1/50, 2%).

All high dose (1,000 mg/kg) male and female mice were dead by week 91 as a result of accidentally being administered 2,800 mg/kg for 3 days during week 91; survival of low dose and control male mice was comparable. Survival of high dose male and dosed female mice may have been inadequate for the detection of late-appearing tumors. No evidence of any carcinogenic effect was found in either low or high dose mice of either sex. An infection of the genital tract was probably responsible for the deaths of 14/22 control and 8/32 low dose female mice before the end of the study.

Cytoplasmic vacuolization was increased in the liver and in the kidney of male and female mice and was considered to be compound related (liver—male: control, 1/50, 2%; low dose, 7/50, 14%; high dose, 47/50, 94%; female: 1/50, 2%; 27/50, 54%; 46/50, 92%; kidney or kidney tubule—male: 0/50; 0/50; 41/50, 82%; female: 0/50; 24/49, 49%; 37/50, 74%).

Under the conditions of these studies, geranyl acetate was not carcinogenic for F344/N rats or $B6C3F_1$ mice of either sex; however, the reduced survival observed in high dose male rats, high dose male mice, and high and low dose female mice lowered the sensitivity of these studies for detecting neoplastic responses in these groups. In male rats the marginal increases of squamous cell papillomas of the skin and tubular cell adenomas of the kidney may have been related to administration of geranyl acetate.

Synonym: 3,7-dimethyl-2,6-octadiene-1-ol acetate

Report Date: October 1987

TR-253 Carcinogenesis Studies of Allyl Isovalerate (CAS No. 2835-39-4) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Allyl isovalerate, a synthetic fragrance and flavoring ingredient in use since the 1950s, may be found in various products at the following concentrations: soap, 30 ppm; detergent, 3 ppm; creams, 15 ppm; perfume, 50 ppm; nonalcoholic beverages, 9 ppm; ice cream, 18 ppm; candy, 22 ppm; baked goods, 15-48 ppm; and gelatins and puddings, 1 ppm. A colorless liquid with an apple-like odor and taste, allyl isovalerate is approved by the U.S. Food and Drug Administration for use in foods. Specific production figures are not available, but U.S. production in 1980 exceeded 1,000 pounds.

Carcinogenesis studies of allyl isovalerate (96% pure) were conducted by administering the test chemical in corn oil gavage to groups of 50 male and 50 female F344/N rats and to groups of 50 male and 50 female $B6C3F_1$ mice at doses of 31 or 62 mg/kg. The doses selected were based on the chemically-induced toxic effects and depressed weight gains obtained from the 13-week studies. Doses were administered five times per week for 103 weeks. Groups of 50 rats and 50 mice of each sex received corn oil by gavage on the same dosing schedule and served as vehicle controls.

Survival and mean body weight gain of rats of each sex and male mice were not adversely affected by the administration of allyl isovalerate. The significantly lower survival ($P = 0.001$) and the lower mean body weight of low-dose female as compared with controls are likely consequences of the high incidence of a genital tract infection in the low-dose females. This infection was probably responsible for the deaths of 11/19 control, 22/33 low-dose, and 13/25 high-dose female mice that died before the end of the study.

Squamous cell papillomas and epithelial hyperplasia of the nonglandular stomach were observed in dosed male mice in the 2-year studies (squamous cell papillomas: 0/50, 1/50, 2%, 3/48, 6%; epithelial hyperplasia: 1/50, 2%, 1/50, 2%, 7/48, 15%). The papillomas occurred with a significant positive trend ($P<0.05$). The incidence of high-dose male mice with squamous cell papillomas of the nonglandular stomach was also higher ($P<0.01$) than the historical rate for vehicle control male B6C3F$_1$ mice in the Bioassay Program (5/881, 0.6%). Forestomach lesions were also observed in female mice: squamous cell papillomas (1/50, 0/50, 2/50) and epithelial hyperplasia of the nonglandular stomach (0/50, 2/50, 3/50). Pancreatic acinar-cell adenomas occurred at higher incidences in the dosed male rats than in the controls (control, 1/50, 2%; low-dose, 4/50, 8%; high-dose, 2/50, 4%). Pancreatic acinar-cell tumors were not observed in female rats. Preputial gland adenomas were observed in increased incidence in low-dose male rats (0/50, 4/50, 8%; $P<0.05$, 1/50, 2%).

Mononuclear-cell leukemias in rats and lymphomas in mice occurred with increased incidences. This consistent dose-response increase among both rats and mice indicates that allyl isovalerate adversely affects the hematopoietic system.

Cholangiofibrosis, nodular regeneration, cirrhosis, focal necrosis, fatty metamorphosis, and cytoplasmic vacuolization were observed at increased incidences in the livers of high-dose male and female rats in the 2-year study. No compound-related nonneoplastic lesions were observed in the mice of either sex. Liver neoplasms were not increased in either dosed rats or mice of either sex. Significant ($P<0.05$) decreases in tumor incidences were observed in male mice for hepatocellular carcinomas (18/50, 6/50, 9/50), for alveolar/bronchiolar adenomas or carcinomas (13/50, 6/50, 5/49), and for follicular-cell adenomas of the thyroid gland (5/47, 0/46, 1/49).

Allyl isovalerate was not mutagenic for *Salmonella typhimurium* (tester strains TA 98, 100, 1535, and 1537) with or without metabolic activation.

Under the conditions of these studies, allyl isovalerate was carcinogenic for F344/N rats and B6C3F$_1$ mice, causing increased incidences of hematopoietic system neoplasms (mononuclear-cell leukemia in male rats and lymphoma in female mice).

Report Date: May 1983

TR-254 Dichloromethane (Methylene Chloride) (CAS: 75-09-2)

Study considered inadequate; no Technical Report will be issued. Dichloromenthane was subsequently studied in F344 rats and B6C3F$_1$ mice by inhallation (See TR-306 published in 1986).

TR-255 Toxicology and Carcinogenesis Studies of 1,2-Dichlorobenzene (*o*-Dichlorobenzene) (CAS No. 95-50-1) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

The major use of 1,2-dichlorobenzene is as an intermediate in the synthesis of several organic compounds (e.g 3,4-dichloroaniline) and in the syntheses of the herbicides propanil, diuron, and neburon. It is used as an industrial solvent for toluene diisocyanate, an additive to degreasing agents, a heat exchange medium, a deodorant for garbage and sewage, an ingredient in paint removers, an engine cleaner and de-inking solvent, and a solvent and intermediate in dye manufacture). 1,2-Dichlorobenzene is also used as an insecticide and a fumigant to control peach tree borers, bark beetles, grubs, and termites and to kill mites and other insects in poultry houses and animal sleeping quarters. Because of its properties as both an insecticide and a solvent, 1,2-dichlorobenzene has been used in low-pressure aerosol formulations of insecticides. Approximately 49 million pounds of 1,2-dichlorobenzene were produced in the United States in 1980. First reported commercial production began in 1921.

In 13-week studies using F344/N rats and B6C3F$_1$ mice, 500 mg/kg of 1,2-dichlorobenzene (>99% pure) decreased survival in male and female mice and female rats when administered in corn oil by gavage five times per week. At this dose, 1,2-dichlorobenzene produced centrolobular necrosis of the liver, hepatocellular degeneration, and depletion of lymphocytes in the thymus and spleen of both sexes of rats and mice. At a dose of 250 mg/kg, necrosis of individual hepatocytes was observed in both sexes of rats and in male mice. Minimal hepatocellular necrosis was observed in a few rats at a dose of 125 mg/kg, but no hepatic alterations were observed in mice at this dose. Renal tubular degeneration was observed in male rats at 500 mg/kg, and multifocal mineralization of the myocardial fibers of the heart and skeletal muscle were seen in mice. The only hematologic changes considered notable were slight decreases in hemoglobin and hematocrit in the 500 mg/kg male and female rats and in red blood cell counts in the 500 mg/kg male rats; no other marked hematological changes were observed in either species.

Two-year toxicology and carcinogenesis studies of 1,2-dichlorobenzene were conducted by administering the test chemical in corn oil gavage five times per week for 103 weeks to groups of 50 male and 50 female F344/N rats and B6C3F$_1$ mice at doses of 60 and 120 mg/kg. Groups of 50 rats and 50 mice of each sex received corn oil by gavage on the same schedule and served as vehicle controls.

Survivals of female rats, male mice, and female mice were comparable to those of the corresponding vehicle controls in the 2-year study, but survival of high dose male rats was ($P<0.001$) shorter than that of the vehicle controls. In this group there were three accidental deaths and five deaths probably due to the gavage process; in

addition aspiration of 1,2-dichlorobenzene in corn oil into the lungs may have been a contributing factor to the deaths of 12 high dose male rats. The 120 mg/kg dose level of 1,2-dichlorobenzene did not affect body weight in rats or mice of either sex or survival of mice or female rats. An increase in tubular regeneration in the kidney of high dose male mice was observed in the 2-year study (control, 8/48, 17%; low dose, 12/50, 24%; high dose, 17/49, 35%). No other compound-related nonneoplastic histological lesions were noted in the 2-year study.

The incidence of pheochromocytoma of the adrenal gland in low dose male rats was elevated ($P<0.05$, life table test) relative to controls (9/50, 16/50, 6/49). However, the incidence in the high dose group was lower than that of the controls and the dose-response trend was not statistically significant. Therefore, the increase in pheochromocytoma in the low dose male rats is not regarded as related to administration of 1,2-dichlorobenzene.

A dose-related increase ($P<0.05$) in malignant histiocytic lymphoma was observed in male mice (control, 0/50, 0%; low dose, 1/50, 2%; high dose, 4/50, 8%) and in female mice (0/49, 0%; 0/50, 0%; 3/49, 6%); however, comparisons of the numbers of animals with all types of lymphomas is considered to be a more appropriate comparison. 1,2-Dichlorobenzene did not increase the incidence of all types of lymphomas (combined) in male mice (8/50, 16%; 2/50, 4%; 4/50, 8%) or female mice (11/49, 22%; 11/50, 22%; 13/49, 27%). Therefore, the increase in histiocytic lymphomas was discounted.

Under the conditions of these two-year gavage studies, there was no evidence of carcinogenicity of 1,2-dichlorobenzene for male or female F344/N rats or $B6C3F_1$ mice receiving 60 or 120 mg/kg per day.

Synonym: *o*-dichlorobenzene

Report Date: October 1985

TR-256 Sodium (2-Ethylhexyl) Alcohol Sulfate (CAS: 126-92-1)

Study considered inadequate; no Technical Report will be issued.

TR-257 Toxicology and Carcinogenesis Studies of Diglycidyl Resorcinol Ether (Technical Grade) (CAS No. 101-90-6) In F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Diglycidyl resorcinol ether (DGRE), a pale, yellow, translucent, amorphous solid at room temperature, is used as a liquid spray epoxy resin, as a diluent in the production of other epoxy resins used in electrical, tooling, adhesive, and laminating applications, and as a curing agent for polysulfide rubber. Approximately 3,000 workers are exposed to DGRE. The quantity of DGRE produced in the United States is not known.

Toxicology and carcinogenesis studies of technical grade diglycidyl resorcinol ether (81% pure) were conducted by administering the chemical in corn oil by gavage to groups of 50 male and 50 female F344/N rats at doses of 25 or 50 mg/kg and to groups of 50 male and female $B6C3F_1$ mice at doses of 50 or 100 mg/kg. A supplemental study of similar design in male and female rats (0 or 12 mg/kg) was started approximately 12 months later because of high mortality in the 50 mg/kg dose groups. Doses were administered five times per week for 103 weeks. Groups of 50 rats and 50 mice of each sex received corn oil by gavage on the same dosing schedule and served as vehicle controls.

Throughout most of the primary study, mean body weights of high dose male and female rats and female mice were lower than those of the corresponding vehicle controls. In the supplemental study, body weights of both sexes of the dosed rats were unaffected by administration of DGRE. Survival of dosed rats of each sex in the primary study was dose related and was shorter ($P<0.001$) than that of the vehicle controls. No high dose male rats and only 1/50 high dose female rats lived to the end of the study. Bronchopneumonia was the most frequent cause of early death among the rats and may have resulted from the animals' aspiration of corn oil containing diglycidyl resorcinol ether. Survival of the dosed male rats in the supplemental study was reduced ($P<0.005$) when compared to controls. There was no significant difference in survival between dosed and control female rats in the supplemental study. Survival of dosed and control mice was comparable but poorer in females, with 20/50 (40%) of the controls, 13/50 (26%) of the low dose, and 10/50 (20%) of the high dose groups alive at the end of 2 years. These early deaths were due to suppurative and necrotizing inflammation of the reproductive tract, possibly caused by a *Klebsiella sp.* infection.

The incidences of rats and mice with hyperkeratosis and hyperplasia of the forestomach were compound related. For rats and mice of each sex, incidences of animals with squamous cell papillomas, squamous cell carcinomas, or both occurred with statistically significant positive trends and the incidences observed in other organs in dosed groups relative to the controls.

An audit of the experimental data was conducted for the 2-year studies of diglycidyl resorcinol ether. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, technical grade diglycidyl resorcinol ether caused hyperkeratosis and hyperplasia of the forestomach in rats and mice. DGRE was carcinogenic for male and female F344/N rats and for male and female $B6C3F_1$ mice, causing both benign and malignant neoplasms of the forestomach.

Synonym: DGRE

Report Date: October 1986

TR-258 Dimethylbenzanthracene (CAS No. 57-97-6)/Tetradecanoyl Phorbol Acetate (CAS No. 20839-11-6) (DMBA/TPA)

Study considered inadequate; no Technical Report will be issued.

TR-259 Carcinogenesis Studies of Ethyl Acrylate (CAS No. 140-88-5) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Ethyl acrylate is a monomer used to produce polymers and copolymers for use in latex paints, textiles, paper coatings, fabric finishes, dirt release agents, and specialty plastics. In 1980, 268 million pounds of ethyl acrylate were produced in the United States of which 209 million pounds were used by the producers and 59 million pounds sold.

Carcinogenesis studies of ethyl acrylate were conducted by administering this test chemical in corn oil by gavage to groups of 50 male and 50 female F344/N rats and B6C3F$_1$ mice at doses of 100 or 200 mg/kg. Ethyl acrylate was administered five times per week for 103 weeks. Groups of 50 rats and 50 mice of each sex received corn oil by gavage on the same schedule and served as vehicle controls.

Survival of dosed male and female rats and mice was comparable with that of the corresponding vehicle controls. There was no evidence of systemic toxicity in the prechronic or in the 2-year studies.

Compound-related increased incidences of hyperkeratosis, inflammation, and hyperplasia of the forestomach were observed in rats and mice in the prechronic as well as 2-year studies. In the 2-year studies, squamous cell papillomas and squamous cell carcinomas of the forestomach occurred at the site of chemical deposition with significant positive trends and increased incidences in dosed groups versus vehicle controls for both sexes of rats and mice. Nonneoplastic and neoplastic forestomach lesion frequencies were related to the concentration of ethyl acrylate in dosing solutions used. Significant negative trends for several common rodent tumors were found in treated animals in the 2-year studies.

Under the conditions of these studies, ethyl acrylate was carcinogenic for the forestomach of F344/N rats and B6C3F$_1$ mice, causing squamous cell carcinomas in male rats and male mice, squamous cell papillomas in male and female rats and male mice, and squamous cell papillomas or carcinomas (combined) in male and female rats and mice. Evidence for carcinogenicity was greater in males than in females. Ethyl acrylate also caused irritation of the forestomach mucosa in male and female rats and mice.

Synonym: 2-propenoic acid, ethyl ester

Report Date: December 1986

TR-260 Tetracholoroethylene (CAS: 127-18-4)

Study considered inadequate: no Technical Report will be issued. Tetrachloroethylene was previously tested in Osborne-Mendel rats and B6C3F$_1$ mice by gavage (See TR-13, reported 1977) and also in F344 rats and B6C3F$_1$ mice by inhalation (See TR-311 published in 1986).

TR-261 Toxicology and Carcinogenesis Studies of Chlorobenzene (CAS No. 108-90-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Chlorobenzene is a colorless, volatile liquid under standard environmental conditions (vapor pressure = 11.8 mm Hg at 25°C, 760 mm Hg). It is used primarily as a solvent (e.g. resins, dyes, pesticides, and perfumes), a degreasing agent, and a chemical intermediate, particularly in the synthesis of nitrobenzenes. Although still considerable, estimates of the yearly production volume of chlorobenzene in the United States indicate declining use in recent years, due to the reduced demand for organochlorine pesticides utilizing chlorobenzene as an intermediate.

Toxicology and carcinogenesis studies of chlorobenzene (>99% pure) were conducted by administering the test chemical in corn oil by gavage to groups of 50 male and 50 female F344/N rats and 50 female B6C3F$_1$ mice at doses of 60 or 120 mg/kg. Groups of 50 male B6C3F$_1$ mice received 30 or 60 mg/kg. Chlorobenzene was administered five times per week for 103 weeks. Groups of 50 rats and 50 mice of each sex received corn oil by gavage on the same schedule and served as vehicle controls, and additional groups of 50 rats and 50 mice of each sex served as untreated controls. The chlorobenzene doses were chosen on the basis of 90-day studies, in which doses 2-fold or greater in excess of the doses used in the 2-year study caused death, hepatocellular necrosis, renal tubular injury, thymic necrosis, or lymphoid or myeloid depletion of bone marrow, spleen or thymus.

Mean body weights of dosed rats and mice were essentially the same or greater than those of the controls during the 2-year studies. Survivals of low dose male rats, dosed female rats, dosed male mice, and dosed female mice were not adversely affected by administration of chlorobenzene. Survival of high dose male rats in the 2-year study was significantly ($P = 0.033$) lower than that of the vehicle controls. No chlorobenzene-induced toxic lesions responsible for this reduction in survival were observed. Based on the prechronic results and on the above data, the doses used in the 2-year study were considered to be adequate for carcinogenicity testing.

Male rats dosed with chlorobenzene exhibited a significant ($P<0.05$) increase in the incidence of animals with neoplastic nodules of the liver (overall incidences: untre-

ated control, 4/50 (8%); vehicle control, 2/50 (4%); low dose, 4/49 (8%); high dose, 8/49 (16)). Increased incidences of hepatocellular carcinomas in male rats or of neoplastic nodules or hepatocellular carcinomas in female rats were not observed. No increased tumor incidences were observed in female rats or in male or female mice.

Under the conditions of these studies, chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in high dose (120 mg/kg/day) male F344/N rats, providing some but not clear evidence of carcinogenicity of chlorobenzene in male rats. Carcinogenic effects of chlorobenzene were not observed in female F344/N rats or in male or female $B6C3F_1$ mice.

Synonyms: monochlorobenzene; chlorobenzol; phenyl chloride; benzene monochloride

Report Date: October 1985

TR-262 1,1,1-Trichloroethane (Methyl Chloroform) (CAS: 71-55-6)

Study considered inadequate; no Technical Report will be issued. 1,1,1-Trichloroethane was previously tested in Osborne-Mendel rats and $B6C3F_1$ mice by gavage (See TR-003, reported 1977).

TR-263 Toxicology and Carcinogenesis Studies of 1,2-Dichloropropane (Propylene Dichloride) (CAS No. 78-87-5) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

1,2-Dichloropropane is a chemical intermediate widely used in the production of tetrachloroethylene and carbon tetrachloride. It is an oil and fat solvent in certain furniture finishes, dry cleaning fluids, and paint removers and has been used to fumigate grain and soil and to control peach tree borers. Approximately 77 million pounds of 1,2-dichloropropane were produced in the United States in 1980. The current 8-hour time-weighted-average concentration to which workers may be exposed to is 75 ppm.

Carcinogenesis studies of 1,2-dichloropropane (>99% pure) were conducted by administering the chemical in corn oil by gavage to groups of 50 female F344/N rats and 50 male and 50 female $B6C3F_1$ mice at doses of 125 or 250 mg/kg body weight and to groups of 50 male F344/N rats at doses of 62 or 125 mg/kg body weight. Doses were administered five times per week for 103 weeks. Vehicle control groups of 50 rats and 50 mice of each sex received an equivalent amount of corn oil by gavage on the same dosing schedule.

Survival was reduced for high dose female rats ($P<0.001$) and for high dose female mice ($P<0.05$) relative to controls; 16/50 high dose female rats and 26/50 high dose female mice survived to the end of the experiment. Survival in the other groups was comparable to the control groups. In female mice, ovarian, uterine, or multiple organ infections may have contributed to the deaths of 5/11 vehicle control, 9/14 low dose, and 14/22 high dose animals that died before the end of the study. There was no evidence of an adverse effect on survival in male rats or in male mice.

Mean body weights of high dose male (-14%) and high dose female (-24%) rats were lower than those of the controls. Low dose rats and all groups of mice had mean body weights comparable to the controls.

High dose female rats had increased incidences of both clear-cell changes and necrosis of the liver; but there was no increase in the incidence of liver tumors in the female rats. There was no treatment-related effects observed in the male rats, other than decreased body weights.

Dose-related increases were observed for adenomas of the liver in both male (control, 7/50; low dose, 10/50; high dose 17/50) and female (1/50, 5/50, 5/50) mice. The increase in the frequency of liver carcinomas supported the evidence that there was a neoplastic response in the mouse liver for both sexes (males: 11/50, 17/50, 16/50, females: 1/50, 3/50, 4/50). Hepatocytomegaly and hepatic necrosis were increased in male mice, but not in female mice.

A dose related increase in adenocarcinomas of the mammary gland was observed in female rats (control, 1/50; low dose, 2/50; high dose, 5/50) with the majority of these tumors being found at the end of the study (1/37, 3%; 2/43, 5%; 4/16, 25%). The incidence of mammary adenocarcinomas was increased when compared to the historical controls for this laboratory (3/150, 2.0%) and for all laboratories combined (11/895, 1.2%). Mammary fibroadenomas were decreased in the high dose treated female rats (15/50, 20/50, 7/50).

The mutagenic activity of 1,2-dichloropropane was marginal. The compound was tested in strains TA100, TA98, TA1537, and TA1535 of *Salmonella typhimurium* in the presence or absence of S9 and no clearly positive response was obtained. Chromosomal aberration and sister chromatid exchange data showed that 1,2-dichloropropane caused increases in both in the absence or presence of S9.

Under the conditions of these 2-year gavage studies, there was no evidence of carcinogenicity for male F344/N rats receiving 62 or 125 mg/kg. For female rats there was *equivocal evidence of carcinogenicity* in that 250 mg/kg 1,2-dichloropropane caused a marginally increased incidence of adenocarcinomas in the mammary gland; these borderline malignant lesions occurred concurrent with decreased survival and reduced body weight gain. There was *some evidence of carcinogenicity* for male and female $B6C3F_1$ mice exposed to 1,2-dichloropropane, as indicated by increased incidences of hepatocellular neoplasms, primarily adenomas.

Synonym: propylene dichloride

Report Date: August 1986

TR-264 Ethoxylated Dodecyl Alcohol (CAS: 9002-92-0)

Study considered inadequate; no Technical Report will be issued.

TR-265 C.I. Acid Yellow 73 (Fluorescein Sodium) (CAS: 518-47-8)

Study considered inadequate; no Technical Report will be issued.

TR-266 Toxicology and Carcinogenesis Studies of Monuron (CAS No. 150-68-5) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Carcinogenesis studies of monuron (greater than 99% pure), a substituted urea herbicide, were conducted by feeding diets containing 0, 750, or 1,500 ppm monuron to groups of 50 F344/N rats of each sex and 0, 5,000, or 10,000 ppm to groups of 50 B6C3F$_1$ mice of each sex for 103 weeks. Survivors then were fed a control diet for 1 week, killed, and examined.

Throughout most of the studies, mean body weights of dosed rats and mice of each sex were lower than those of the controls. Survival rates of low dose female rats and high dose male and female mice were increased relative to those of the controls.

In 13-week toxicity studies, the lympho/hematopoietic system of rats and mice was the primary site affected. The lymphoid depletion found in these animals was not seen in rats or mice surviving to the end of the 104-week studies.

Nonneoplastic changes associated with the long-term administration of monuron to rats included renal tubular cell cytomegaly, mainly involving the proximal convoluted tubules in male and female rats, and dose-related hepatic cytoplasmic changes in male rats.

In the 104-week study, the kidneys and liver of male rats were the primary tissues affected. Long-term administration of monuron was associated with an increase in renal tubular cell adenomas (control, 0/50; low dose, 2/50; high dose, 7/50) and renal tubular cell adenocarcinomas (0/50; 1/50; 8/50). Administration of monuron to male rats was associated with increased incidences of neoplastic nodules of the liver (1/50; 6/49; 7/50) and of neoplastic nodules or carcinomas (combined) of the liver (1/50; 6/49; 9/50).

Dosed male and female rats had decreased incidences of mononuclear cell leukemia; dosed male rats had lower incidences of pheochromocytomas of the adrenal glands and C-cell carcinomas of the thyroid gland; dosed female rats had reduced incidences of mammary gland fibroadenomas.

In male mice, dose-related decreases occurred in the incidences of hepatocellular carcinomas (6/50; 5/49; 2/50) and hepatocellular adenomas or carcinomas (12/50; 8/49; 6/50); incidences of hepatocellular tumors in low dose female mice were reduced in dosed female mice (16/50; 8/50; 7/50).

Monuron was not mutagenic in Salmonella strains TA98, TA100, TA1535, or TA1537 in the presence or absence of Aroclor 1254-induced rat liver S9. Monuron did induce chromosomal aberrations and sister chromatid exchanges in cultured Chinese hamster ovary cells.

The data, documents and pathology materials from the 2-year studies of monuron have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenicity* for male F344/N rats in that monuron caused increased incidences of tubular cell adenocarcinomas of the kidney, tubular cell adenomas of the kidney, and neoplastic nodules or carcinomas (combined) of the liver. Monuron induced cytomegaly of the renal tubular epithelial cells in both male and female F344/N rats. There was *no evidence of carcinogenicity* for female F344/N rats or for male or female B6C3F$_1$ mice.

Synonyms and Trade Names: *N'*-(4-chlorophenyl)-*N,N*-dimethylurea; 1,1-dimethyl-3-(*p*-chlorophenyl)urea; CMU; Karmex Monuron Herbicide; Telvar

Report Date: August 1988

TR-267 Toxicology and Carcinogenesis Studies of Propylene Oxide (CAS no. 75-56-9) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

Propylene oxide is a volatile, colorless liquid used as an intermediate in the production of polyether polyols, polyurethane foams, and unsaturated polyester resins and also as a fumigant for sterilizing a variety of materials ranging from plastic medical instruments to foodstuffs. In the United States, propylene oxide is registered as a fumigant for packaged dried prunes and glacé fruits such as candied cherries and as an insecticidal and fungicidal fumigant for bulk quantities of cocoa, gums, and processed spices.

The 2-year carcinogenesis studies of propylene oxide (greater than 99.9% pure) were conducted by exposing groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex to air containing propylene oxide at concentrations of 0 (chamber control), 200, or 400 ppm for 6 hours per day, 5 days per week, for 103 weeks.

The survival of rats exposed to propylene oxide was comparable with that of the controls; terminal body

weights were lower than those of the controls for high dose males (8%) and high dose females (6%). Survival of exposed male and female mice decreased relative to that of the controls (male: control, 42/50; low dose, 34/50; high dose, 29/50; female: 38/50; 29/50; 10/50), but the difference was significant only for animals in the high dose groups. High dose female mice had a mean terminal body weight 22% below that of the controls.

The respiratory epithelium of the nasal turbinates was one of the primary tissues affected in male and female rats; exposure-related increases occurred in the incidences of suppurative inflammation, epithelial hyperplasia, and squamous metaplasia. Papillary adenomas, involving the respiratory epithelium and underlying submucosal glands of the nasal turbinates, were observed in three female rats and two male rats exposed to propylene oxide at 400 ppm. The incidence of adenomas in females was significant by the trend tests.

The proportions of high dose female rats with C-cell adenomas and with C-cell carcinomas of the thyroid gland were increased, but only the combined incidence of these tumors was significant (2/45; 2/35; 7/37). These tumors were not considered to be related to exposure to propylene oxide because there was no other evidence for C-cells' being a target tissue and because there was no increase in C-cell hyperplasia.

The combined incidences of female rats with endometrial stromal polyps and endometrial stromal sarcomas of the uterus were significantly increased in the dosed groups (3/49; 12/50; 10/47). However, the occurrence of these lesions in the dosed groups was similar to the average (306/1,502, 20%) seen in untreated controls in NTP carcinogenesis studies, and hence this increase was not regarded as being related to exposure to propylene oxide.

The respiratory epithelium of the nasal turbinates was also one of the primary tissues affected in male and female mice; exposure-related increases occurred in the incidences of inflammation, and squamous metaplasia was observed in one low dose male and two high dose female mice. One squamous cell carcinoma and one papilloma occurred in the nasal cavity of different high dose male mice, and two high dose female mice had adenocarcinomas of the nasal cavity. The endothelial cells of the submucosal vascular plexus in the nasal turbinates also appeared to be a major site affected in high dose male mice. Three high dose male and three high dose female mice had a saccular dilation (classified as angiectasis) of submucosal turbinate vessels. Further, hemangiomas were seen in the nasal cavity of 5/50 high dose male mice and 3/50 high dose female mice, and hemangiosarcomas were found in the nasal cavity of 5/50 high dose male mice and 2/50 high dose female mice. The increased incidences of hemangiomas in males and females and of hemangiosarcomas in males were statistically significant. Vascular tumors were not present in the nasal turbinates of any low dose or control mice.

Under the conditions of these studies, there was *some evidence of carcinogenicity* for F344/N rats, as indicated by increased incidences of papillary adenomas of the nasal turbinates in male and female rats exposed to propylene oxide at 400 ppm. For male and female $B6C3F_1$ mice, there was *clear evidence of carcinogenicity*, as indicated by increased incidences of hemangiomas or hemangiosarcomas of the nasal turbinates at 400 ppm. In the respiratory epithelium of the nasal turbinates, propylene oxide also caused suppurative inflammation, hyperplasia, and squamous metaplasia in rats and inflammation in mice.

Report Date: March 1985

TR-268 Sodium Dodecyl Sulfate (CAS: 151-21-3)

Study considered inadequate; no Technical Report will be issued.

TR-269 Toxicology and Carcinogenesis Studies of Telone II® (Technical-Grade 1,3-Dichloropropane [CAS No. 542-75-6] Containing 1.0% Epichlorohydrin as a Stabilizer) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of Telone II® (a soil fumigant containing approximately 89% *cis*- and *trans*-1,3-dichloropropene, 2.5% 1,2-dichloropropane, 1.5% of a trichloropropene isomer, and 1.0% epichlorohydrin) were conducted by administering the commercial-grade formulation in corn oil by gavage to groups of 52 male and 52 female F344/N rats at doses of 0, 25, or 50 mg/kg and to groups of 50 male and 50 female $B6C3F_1$ mice at doses of 0, 50, or 100 mg/kg. Doses were administered three times per week for 104 weeks. Ancillary studies were conducted in which dose groups containing five male and five female rats were killed after receiving Telone II® for 9, 16, 21, 24, or 27 months.

Mean body weights of high dose male rats were about 5% lower than those for the vehicle control and low dose male rats; no differences in body weights were observed among groups of female rats. Survival was comparable for the different groups of male and female rats. For male and female mice, the dosed groups initially weighed 6%-22% less than did the vehicle controls; the weight differential decreased to 5%-9% by the end of the studies. Twenty-five vehicle control male mice died during weeks 48-51 from suppurative inflammation of the heart (myocarditis). At the end of the studies, the survival of male mice was as follows: vehicle control, 8/50; low dose, 28/50; high dose 31/50. Survival of female mice was lower ($P<0.05$) in the high dose group than in the vehicle controls (46/50; 45/50; 36/50).

The primary organs affected were the forestomach (rats and mice), urinary bladder (mice), lung (mice), and liver (rats). Compound-related nonneoplastic lesions

included basal cell or epithelial hyperplasia of the forestomach (rats and mice), epithelial hyperplasia of the urinary bladder (mice), and kidney hydronephrosis (mice). Neoplastic lesions associated with administration of Telone II® included squamous cell papillomas of the forestomach (male rats: 1/52; 1/52; 9/52; female rats: 0/52; 2/52; 3/52; female mice: 0/50; 1/50; 2/50), squamous cell carcinomas of the forestomach (male rats: 0/52; 0/52; 4/52; female mice: 0/50; 0/50; 2/50), transitional cell carcinomas of the urinary bladder (female mice: 0/50; 8/50; 21/48), alveolar/bronchiolar adenomas (female mice: 0/50; 3/50; 8/50), and neoplastic nodules of the liver (male rats: 1/52; 6/52; 7/52).

Although the study in male mice was considered inadequate due to the deaths at weeks 48-51 of 25/50 vehicle control animals, 2/50 of the high dose males had transitional cell carcinomas of the urinary bladder. Furthermore, increases were seen in the incidences of alveolar/bronchiolar neoplasms of the lung (1/50; 13/50; 12/50) and of squamous cell papillomas of the forestomach (0/50; 2/50; 3/50). These findings plus the finding of nonneoplastic lesions in two of these organs (basal cell or epithelial hyperplasia of the forestomach: 0/50; 0/50; 4/50; epithelial hyperplasia of the urinary bladder: 0/50; 9/50; 18/50) suggest that Telone II® may have been responsible for the development of these lesions in male mice.

Development of lesions of the forestomach (basal cell hyperplasia and squamous cell papilloma) was observed to be time dependent. The results of the scheduled kills supported the findings of the carcinogenesis studies. When the results from the scheduled kills at all time points were pooled with those of the 24-month carcinogenesis studies, the incidences were as follows: basal cell or epithelial hyperplasia of the forestomach—male rats: 3/77; 13/77; 31/77; female rats: 1/75; 5/77; 35/77; squamous cell papilloma of the forestomach—male rats: 1/77; 1/77; 13/77; female rats: 0/75; 2/77; 8/77; neoplastic nodules of the liver—male rats: 1/77; 6/76; 8/77; female rats: 6/75; 8/77; 12/77.

cis- and *trans*-1,3-Dichloropropane are the principle components (89%) in Telone II®, but the 1.0% epichlorohydrin, a direct-acting mutagen and carcinogen added as a stabilizer, may have influenced the development of the forestomach lesions.

1,3-Dichloropropene was mutagenic in *Salmonella typhimurium* strains TA98, TA100, and TA1535 without metabolic activation and in TA100 and TA1535 with metabolic activation by Aroclor-induced male Sprague-Dawley rat and Syrian hamster liver S9. No mutagenic response was seen in TA1537. Sex-linked recessive lethal mutations were observed in *Drosophila melanogaster*, and 1,3-dichloropropene did not induce reciprocal translocations in *D. melanogaster*.

A data audit was conducted on the Telone II® 2-year carcinogenesis studies in rats and mice and the ancillary studies in rats. Except for the already known problem of survival of male vehicle control mice, no other discrepancies or problems that would compromise the validity of the findings or alter the interpretations of these studies were found.

Under the conditions of these gavage studies, there was *clear evidence of carcinogenicity* for male F344/N rats, as indicated by Telone II®-related increased incidences of squamous cell papillomas and carcinomas of the forestomach, as well as an increased incidence of neoplastic nodules of the liver. In female F344/N rats, there was *some evidence of carcinogenicity* because Telone II® caused an increased incidence of squamous cell papillomas of the forestomach. The experiment in male B6C3F$_1$ mice was considered to be an *inadequate study of carcinogenicity* because of reduced survival in the vehicle control group. However, there was some indication in the male mice of Telone II®-related increases of transitional cell carcinomas of the urinary bladder, squamous cell papillomas of the forestomach, and alveolar/bronchiolar adenomas and carcinomas of the lung. There was *clear evidence of carcinogenicity* for female B6C3F$_1$ mice, since Telone II® caused increased incidences of transitional cell carcinomas of the urinary bladder; Telone II® also increased the incidences of alveolar/bronchiolar adenomas of the lung and of squamous cell papillomas or carcinomas of the forestomach in the female mice. Telone II®-related nonneoplastic lesions included basal cell or epithelial cell hyperplasia in the forestomach of male and female and male and female mice and epithelial hyperplasia of the urinary bladder in male and female mice.

Synonyms: *cis,trans*-1,3-dichloropropene, D-D® Soil Fumigant; Telone® Soil Fumigants; Vorlex® Soil Fumigants

Report Date: May 1985

TR-270 Gilsonite (CAS: 12002-43-6)

Study considered inadequate; no Technical Report will be issued.

TR-271 Toxicology and Carcinogenesis Studies of HC Blue No. 1 (CAS No. 2784-94-3) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Toxicology and carcinogenesis studies of HC Blue No. 1 (97% pure), a semipermanent hair dye, were conducted by administering the test chemical in feed for 103 weeks to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex. The dietary concentrations used were 0, 1,500, or 3,000 ppm for rats and male mice and 0, 3,000, or 6,000 ppm for female mice. These concentrations were selected on the basis of results from single-administration gavage studies and 14-day and 13-week feed studies.

The survival of male and female rats and male mice was not affected by administration of HC Blue No. 1. Survival of high dose female mice was reduced ($P<0.05$); the early deaths in this group are believed to have been caused by hepatocellular carcinomas. Body weights of

high dose rats and dosed mice were lower than those of the respective control groups; female rats and mice were more affected than were males.

Administration of HC Blue No. 1 produced significant positive trends in the incidences of male rats with hepatocellular neoplastic nodules/carcinomas (neoplastic nodules: control, 0/49; low dose, 0/50; high dose, 3/50; neoplastic nodules/carcinomas: 1/49; 0/50; 6/50). In male and female mice, both doses of HC Blue No. 1 increased the incidences of hepatocellular carcinoma (male: 11/50; 20/50; 30/50; female: 1/50; 24/48; 47/49) and the low doses increased the incidences of hepatocellular adenomas (male: 4/50; 17/50; 10/50; female: 2/50; 11/48; 4/49).

HC Blue No. 1 produced dose-related increases in the incidences of proliferative lesions of the lungs (adenomatous hyperplasia and alveolar/bronchiolar adenomas or carcinomas) in female rats (hyperplasia: 2/50; 5/49; 8/50; adenoma/carcinoma: 1/50; 3/49; 7/50).

In male mice, HC Blue No. 1 at the 6,000-ppm dose increased the incidences of thyroid gland follicular cell hyperplasia and adenomas (hyperplasia: 3/47; 7/49; 14/50; adenoma: 0/47; 0/49; 5/50).

HC Blue No. 1 was mutagenic in strains TA97, TA98, and TA100 of *Salmonella typhimurium* in the presence or absence of Aroclor-induced male Sprague-Dawley rat or Syrian hamster liver S9; HC Blue No. 1 was negative in strain TA1535. HC Blue No. 1 was mutagenic in the absence of activation in the L5178Y/TK$^{+/-}$ mouse lymphoma assay and induced unscheduled DNA synthesis in rat hepatocytes in vitro.

An audit of the experimental data was conducted for these carcinogenesis studies on HC Blue No. 1. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these feed studies, there was *equivocal evidence of carcinogenicity* in male F344/N rats, since HC Blue No. 1 caused a marginal increase in the incidence of hepatocellular neoplastic nodules/carcinomas. For female F344/N rats, there was *some evidence of carcinogenicity* in that HC Blue No. 1 induced increased incidences of alveolar/bronchiolar neoplasms. There was *clear evidence of carcinogenicity* of HC Blue No. 1 for male and female B6C3F$_1$ mice as shown by increased incidences of hepatocellular carcinomas. The incidences of follicular cell adenomas of the thyroid gland were also increased in male mice receiving HC Blue No. 1.

Synonym: 2,2'((4-(methylamino)-3-nitrophenyl) imino)bis (ethanol)

Report Date: August 1985

TR-272 Toxicology and Carcinogenesis Studies of Propylene (CAS No. 115-07-1) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

Propylene is used as a starting material in the production of polypropylene plastics and various other chemicals, including acrylonitrile, isopropyl alcohol, propylene oxide, butyraldehyde, cumene, dodecane, nonene, and allyl chloride. The major derivatives are polypropylene (25%), acrylonitrile (15%), isopropyl alcohol (10%), and propylene oxide (10%). It is also a valuable feed-stock chemical for the production of gasoline. Other miscellaneous applications include use as a starting material for polymerization reactions to form vinyl chloride copolymers and low-molecular-weight homopolymers that are used as additives in lubricating oils and in the manufacture of hydroquinone. The chemical is also used as an aerosol propellant or component. The major end uses of propylene are in the production of fabricated plastics (50%) and fibers (15%).

Toxicology and carcinogenesis studies of propylene (greater than 99% pure) were conducted by exposing groups of 50 F344/N rats and 49 or 50 B6C3F$_1$ mice of each sex to propylene in air by inhalation at concentrations of 5,000 or 10,000 ppm, 6 hours per day, 5 days per week, for 103 weeks. Other groups of 50 rats and 50 mice of each sex in chambers received air only on the same schedule and served as chamber controls. The highest concentration of propylene that was considered safe for these studies was 10,000 ppm because of the risk of explosion that can occur at higher concentrations.

The survival of exposed and control rats and mice was comparable. Throughout most of the studies, mean body weights of exposed male and female rats were slightly lower (0%-5%) than those of the controls, but the decrements were not concentration related. After week 59 of the study, mean body weights of 10,000-ppm male mice were usually slightly lower (5%) than those of the controls, whereas those in other exposed groups of male and female mice were generally comparable with those of the controls. No compound-related adverse clinical signs were observed in either species.

An increased incidence of squamous metaplasia of the nasal cavity was observed in female rats exposed at the 5,000-ppm and 10,000-ppm concentrations (control, 0/49; low, 15/50; high, 6/50) and in male rats exposed at 5,000 ppm (2/50; 19/50; 7/50). Epithelial hyperplasia of the nasal cavity was increased in female rats exposed at the 10,000-ppm concentration (0/49; 4/50; 9/50); the incidences in male rats were 2/50, 2/50, and 5/50. Inflammation of the nasal cavity, characterized by an influx of lymphocytes, macrophages, and granulocytes into the submucosa and by granulocytes into the lumen, occurred at increased incidences in low concentration and high concentration male rats and in high concentration female rats. Chronic focal inflammation of the kidneys occurred at an increased incidence in low concentration and high concentration mice of each sex.

Hemangiosarcomas were found in one low dose male mouse (liver), two high dose male mice (spleen), and three high dose female mice (subcutis, spleen, and uterus). Hemangiomas were found in one low dose and in one high dose female mouse (liver). Vascular tumors were not found in control mice of either sex. The low incidences of vascular tumors and their occurrence in a variety of

organs suggest that they are not related to administration of propylene.

The occurrence of uterine endometrial stromal polyps in female mice showed a positive trend ($P<0.05$; 0/47; 0/47; 3/48); the incidence in the 10,000-ppm group was not significantly greater than that in the concurrent control group, but the incidence was higher than the mean historical control rate (22/2,411, 0.9%) and was within the range (0%-6%) observed in studies throughout the Carcinogenesis Program. The occurrence of endometrial stromal polyps in three high concentration female mice was not considered to be clearly related to exposure to propylene.

The incidence of male mice with alveolar/bronchiolar adenomas or carcinomas (combined) occurred with a negative trend ($P<0.05$; 16/50; 4/49; 7/50), and the reduced incidences in both exposed groups were less ($P<0.05$) than that in the control group. The control incidence of these tumors in an inhalation study conducted concurrently at the same laboratory was similar (15/50), suggesting a possible exposure-related decrease. The biologic significance of this decrease in male mice is difficult to assess; the incidences seen in these control and exposed animals are within the range of incidences (2%-34%; mean, 16.7%) observed in control male mice in other studies throughout the Carcinogenesis Program.

An audit of the experimental data was conducted for these carcinogenesis studies on propylene. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these studies, there was *no evidence of carcinogenicity* in male and female F344/N rats or in male and female $B6C3F_1$ mice exposed to propylene by inhalation at concentrations of 5,000 or 10,000 ppm for 103 weeks. In the nasal cavity, propylene induced squamous metaplasia of the respiratory epithelium in male and female rats and epithelial hyperplasia in female rats.

Synonyms: propene; methylethylene; methylethene

Report Date: November 1985

TR-273 Toxicology and Carcinogenesis Studies of Trichloroethylene (CAS No. 79-01-6) in Four Strains of Rats (ACI, August, Marshall, Osborne-Mendel) (Gavage Studies)

Trichloroethylene is an industrial solvent used primarily for vapor degreasing and cold cleaning. It was selected for study because of its industrial use and for potential for human exposure. (An estimated 3.5 million workers are exposed to trichloroethylene.) In an earlier study trichloroethylene (stabilized with epichlorohydrin and 1,2-epoxybutane) administered by gavage caused hepatocellular carcinomas in male and female $B6C3F_1$ mice. Trichloroethylene administration did not increase the incidence of tumors in male or female Osborne-Mendel rats. However, the survival of dosed rats was reduced, thereby compromising the sensitivity of the study to detect a carcinogenic effect.

The studies described in this report were conducted to compare the sensitivities of four strains of rats (ACI, August, Marshall, and Osborne-Mendel) to diisopropylamine-stabilized trichloroethylene. The results of the present studies demonstrate that long-term administration of trichloroethylene produces nephrotoxicity in four strains of rats and that the susceptibilities of these strains to the nephrotoxic effects of the chemical are similar. Because of chemically induced toxicity, reduced survival, and incomplete documentation of the experimental data, the studies are considered inadequate for either comparing or assessing trichloroethylene-induced carcinogenesis in these strains of rats.

Toxicology and carcinogenesis studies of trichloroethylene (more than 99% pure, stabilized with 8 ppm diisopropylamine) were conducted by administering the chemical in corn oil gavage at doses of 0, 500, or 1,000 mg/kg per day, 5 day per week, for 103 weeks to groups of 50 male and 50 female ACI, August, Marshall, and Osborne-Mendel rats. The doses were selected on the basis of results from 13-week gavage studies in which groups of 10 male and 10 female ACI, August, and Marshall rats received daily doses or trichloroethylene (male: 125-2,000 mg/kg; female: 63-1,000 mg/kg). Doses for Osborne-Mendel rats were selected to conform with doses used in an earlier carcinogenicity study in that strain (NCI TR-2).

In the 13-week studies, male ACI and August rats receiving 2,000 mg/kg trichloroethylene and male and female Marshall rats receiving 1,835 mg/kg had final mean body weights 12%-17% lower than those of the vehicle controls. All other dose groups had body weights comparable to those of the vehicle controls. Three male August rats dosed with 2,000 mg/kg died. Histopathologic evaluation of tissues revealed no lesions attributable to trichloroethylene administration in the 13-week studies. This absence of histopathologic findings did not accurately predict the nephrotoxic effects of long-term administration of trichloroethylene to rats.

Body Weight and Survival in the Two-Year Studies: In the 2-year studies, all dosed groups exhibited some reduction in mean body weights relative to the vehicle controls. Survival relative to vehicle controls was significantly reduced in 7/16 dosed groups (see page 6 of the Technical Report). Also, the survival of high dose male Marshall rats was reduced by a large number of accidental deaths. Nephrotoxicity, reduced survival, and central nervous system toxicity (characterized by sedation, loss of consciousness, tremors, and convulsions) showed that the doses of trichloroethylene selected for the 2-year studies were too high.

Renal Effects in the Two-Year Studies: Trichloroethylene caused tubular cell cytomegaly in 82%-100% of all dosed animals. In addition, trichloroethylene produced toxic nephropathy (which was distinguishable from age-related nephropathy) in 17%-80% of the dosed animals. Cytomegaly, karyomegaly, or toxic nephropathy was not

found in untreated or vehicle control animals. Trichloroethylene administration was also associated with increased incidences of renal tubular cell adenomas and adenocarcinomas. The incidences of renal lesions are shown in the following table (see page 7 of Technical Report).

Other Pathologic Effects in the Two-Year Studies: An increased incidence of interstitial cell tumors of the testis was observed in high dose male Marshall rats (untreated control, 16/46; vehicle control, 17/46; low dose, 21/48; high dose, 32/48; $P = 0.002$). The incidences of pheochromocytomas of the adrenal gland were significantly reduced in male ACI, female August, female Marshall, and male and female Osborne-Mendel rats.

Genetic Toxicology: Trichloroethylene did not cause mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation. In Chinese hamster ovary cells, trichloroethylene did not induce chromosomal aberrations; the results for sister chromatid exchanges were considered positive. Trichloroethylene was mutagenic to mouse L5178Y lymphoma cells in the presence of rat liver S9.

Data Audit: Audits of the experimental data for these 2-year studies of trichloroethylene were conducted by the National Toxicology Program (see Appendix Q of the Technical Report). The results of the audits revealed evidence that the doses of trichloroethylene were too high. In addition, there was insufficient documentation of animal breeding, clinical observations, environmental conditions, and analytical chemistry data. Also, individual animal identification was not always verifiable.

Conclusions: Under the conditions of these 2-year gavage studies of trichloroethylene in male and female ACI, August, Marshall, and Osborne-Mendel rats, trichloroethylene administration caused renal tubular cell cytomegaly and toxic nephropathy in both sexes of the four strains. However, these are considered to be *inadequate studies of carcinogenic activity* because of chemically induced toxicity, reduced survival, and deficiencies in the conduct of the studies. Despite these limitations, tubular cell neoplasms of the kidney were observed in rats exposed to trichloroethylene and interstitial cell neoplasms of the testis were observed in Marshall rats exposed to trichloroethylene.

Synonyms: acetylene trichloride; 1-chloro-2,2-dichloroethylene; 1,1-dichloro-2-chloroethylene; ethinyl trichloride; ethylene trichloride; 1,1,2-trichloroethylene; trichloroethene

Trade names of formulations: Algylen; Anamenth; Benzinol; Blacosolv; Blancosolv; Cecolene; Chlorilen; Chlorylea; Chorylen; Circosolv; Crawhaspol; Densinfluat; Dow-Tri; Dukeron; Fleck-Flip; Flock Flip; Fluate; Gemalgene; Germalgene; Lanadin; Lethurin; Narcogen; Narkogen; Narkosoid; Nialk; Perma-A-Chlor; Perm-A-Clor; Petzinol; Philex; Threthylen; Threthylene; Trethylene; Tri; Triad; Trial; Triasol; Trichloran; Trichloren; Triclene; Tri-Clene; Trielene; Trielin; Triklone; Trilen; Trilene; Triline; Trimar; Triol; TRI-plus; TRI-plus M; Vestrol; Vitran; Westrosol

Report Date: April 1988

Note: Trichloroethylene was previously tested in Osborne-Mendel rats and $B6C3F_1$ mice by gavage (See TR-2, reported 1977) and also in F344 rats and $B6C3F_1$ mice by gavage (See TR-243, reported 1990).

TR-274 Toxicology and Carcinogenesis Studies of Tris(2-ethylhexyl)phosphate (CAS No. 78-42-2) In F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Tris(2-ethylhexyl)phosphate is one of a family of triakyl phosphates that have been widely used as fire retardants and plasticizers. Another triakyl phosphate, tris(2,3-dibromopropyl)phosphate (Tris-BP), once used as a flame retardant in children's sleepwear, has been shown to be carcinogenic, but tris(2-ethylhexyl)-phosphate has not been previously studied. Tris(2-ethylhexyl)phosphate, a clear, viscous liquid, is used as a component of vinyl stabilizers, grease additives, and flame-proofing compositions; however, it is used primarily as a plasticizer for vinyl plastic and synthetic rubber compounds. In 1974, approximately 3 million pounds of tris(2-ethylhexyl)phosphate was produced in the United States; imports during that year were negligible. Substantial human exposure probably occurs during production of tris(2-ethylhexyl)phosphate and during the manufacture and use of products containing it, but data on the magnitude of exposure are not available.

Two-year toxicology and carcinogenesis studies of tris(2-ethylhexyl)phosphate were conducted by administering the test chemical in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 male and 50 female F344/N rats and $B6C3F_1$ mice. Male rats received doses of 2,000 or 4,000 mg/kg body weight, female rats received 1,000 or 2,000 mg/kg, and male and female mice received 500 or 1,000 mg/kg. Fifty vehicle control animals of each sex and species received 10 ml/kg body weight (rats) or 3.3 ml/kg (mice) corn oil by gavage on the same schedule.

Inflammation of the gastric mucosa in mice and mild weight depression in rats and mice were the only dose-related effects observed in the preliminary studies. In the 2-year studies, survival rates and mean body weight gains of dosed female rats and dosed mice were comparable to those of their perspective controls. Survival rates of dosed male rats were comparable to that of the vehicle controls, but body weight gains were depressed. One nonneoplastic lesion, follicular cell hyperplasia of the thyroid, was observed at increased incidences in dosed male and female mice.

Two compound-related increased incidences of neoplasms could not be discounted. In male rats, the incidence of pheochromocytoma of adrenal glands increased with dose (2/50, 4%; 9/50, 18%; 12/50, 24%). There were also two additional malignant pheochromocytomas in the

high dose group. However, the incidence of adrenal pheochromocytoma in vehicle controls of this study (2/50, 4%) was low compared with the 25% incidence observed in two previous studies in this laboratory or the overall historical incidence of 18% observed throughout the Program, and thus the evidence of carcinogenicity was considered to be equivocal. In female mice, the incidence of hepatocellular carcinoma (0/48; 4/50; 7/50) in high dose animals (1,000 mg/kg) was significantly increased relative to that of the vehicle controls.

Decreased incidences were observed for acinar cell adenomas of the pancreas in dosed male rats (14/50, 28%; 5/48, 10%; 2/49, 4%) and for fibroadenomas of the mammary glands in low dose female rats (11/50, 22%; 2/50, 4%; 7/50, 14%). Hemangiosarcomas of the circulatory system in male mice (7/50, 14%; 0/50; 1/49, 2%) and lymphomas of the hematopoietic system in female mice (14/49, 29%; 10/50, 20%; 6/50, 12%) were decreased compared with vehicle controls. A decrease in the incidence of lymphomas and an increased incidence of carcinomas of the liver in female mice (both seen in this study) were observed in studies of di(2-ethylhexyl)adipate. Increased incidences of liver carcinomas and decreased incidences of mammary fibroadenomas were observed also in female rats in the di(2-ethylhexyl)phthalate studies. A possible link among these three chemicals may be metabolic conversion to 2-ethylhexanol.

Tris(2-ethylhexyl)phosphate was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of 9000 x g (S9) fractions from Aroclor 1254-induced Sprague-Dawley rat or Syrian hamster liver.

An audit of the experimental data from these carcinogenesis studies was conducted by the National Toxicology Program. No data discrepancies were found that significantly influenced the final interpretations of these experiments.

Under the conditions of these studies, a comparison of concurrent and historical controls indicated that there was *equivocal evidence of carcinogenicity* in male F344/N rats receiving 2,000 and 4,000 mg/kg tris(2-ethylhexyl)phosphate, as evidenced by increased incidences of pheochromocytomas of the adrenal glands. There was *no evidence of carcinogenicity* in female F344/N rats or in male B6C3F$_1$ mice receiving tris(2-ethylhexyl)phosphate. There was *some evidence of carcinogenicity* in female B6C3F$_1$ mice that received 1,000 mg/kg tris(2-ethylhexyl)phosphate, as shown by an increased incidence of hepatocellular carcinoma. Tris(2-ethylhexyl)phosphate was associated with increased incidences of follicular cell hyperplasias of the thyroid gland in male and female B6C3F$_1$ mice.

Synonyms and Trade Names: TOF; trioctyl phosphate; phosphoric acid tri(2-ethylhexyl) ester; Flexol® TOF; Kronitex®

Report Date: August 1984

TR-275 Toxicology and Carcinogenesis Studies of 2-Chloroethanol (Ethylene Chlorohydrin) (CAS No. 107-07-3) in F344/N Rats and Swiss CD-1 Mice (Dermal Studies)

Toxicology and carcinogenesis studies of 2-chloroethanol (99% pure), an industrial chemical and an intermediate in the synthesis of ethylene oxide, were conducted by dermal application of 2-chloroethanol dissolved in 70% ethanol:30% water (v/v) solutions to groups of 50 F344/N rats of each sex at doses of 0, 50, or 100 mg/kg for 103 weeks or to groups of 50 Swiss CD-1 mice of each sex at doses of 0, 7.5, or 15 mg per animal for 104 weeks (0, 253, or 630 mg/kg at week 1; 0, 180, 411 mg/kg at week 100). The control groups received skin applications of the vehicle; the mouse studies also included untreated control groups of 50 male and 50 females.

2-Chloroethanol solutions were applied to the clipped interscapular area of the animals once daily, 5 days per week for the test period. Rats received a volume of 0.18-0.22 ml of solution; mice received 0.10 ml of solution. In the 13-week studies, mortality was observed in male and female rats receiving 20 mg per day and higher. In the 104-week studies, the survival of high dose male mice was lower ($P<0.05$) than that of the vehicle controls (vehicle control, 26/50; 7.5 mg, 16/50; 15 mg, 12/50). Body weights of dosed mice were unaffected by 2-chloroethanol. The survival and body weight gain data suggest that the male and female rats and female mice could have tolerated a higher dose of 2-chloroethanol. Male mice probably could not have tolerated a higher dose than was applied to the skin. Seven high dose male mice died within 3 days of the start of dosing; all of these had inflammation at the site of dermal application. Five also had ulceration at the site of dermal application, and five had lung congestion, inflammation, or hemorrhage.

Marginal increases were found in the incidence of lymphomas or leukemias (combined) as well as in the incidence of alveolar/bronchiolar adenomas or carcinomas (combined) in low dose male mice. Since there was no dose-related trend for these tumor incidences and because the increases were observed in only one sex, the increases were not considered to be related to the dermal application of 2-chloroethanol.

2-Chloroethanol was mutagenic in *Salmonella typhimurium* strains TA100 and TA1535 (but not TA1537 or TA98) in either the presence or the absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. 2-Chloroethanol did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*.

An audit of the experimental data was conducted for these 2-year studies. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenicity* of 2-chloroethanol for male and female F344/N rats given 50 or

100 mg/kg per day or for male and female Swiss CD-1 mice given 7.5 or 15 mg per animal per day.

Synonyms: ethylene chlorohydrin; chloroethanol; glycol chlorohydrin; β-chloroethanol

Report Date: November 1985

TR-276 Toxicology and Carcinogenesis Studies of 8-Hydroxyquinoline (CAS No. 148-24-3) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Carcinogenesis studies of 8-hydroxyquinoline (99% pure), a metal chelator and antimicrobial agent, were conducted by administering the test chemical in feed to groups of 50 male and 50 female F344/N rats and B6C3F$_1$ mice at concentrations of 0, 1,500, or 3,000 ppm for 103 weeks. These concentrations were selected because the chemical at higher concentrations resulted in reduced feed consumption, decreases in mean body weights, and deaths in the 15-day and 13-week studies. The average daily doses were estimated to be 73 and 143 mg/kg for male rats, 89 and 166 mg/kg for female rats, 217 and 396 mg/kg for male mice, and 349 and 619 mg/kg for female mice.

Survival of dosed male and female rats and mice in the 2-year studies was comparable to that of the corresponding controls. The high dose rats and mice of each sex exhibited slight decreases in mean body weights and decreased feed consumption.

Compound-related gross or microscopic pathologic effects were not observed in either species in the 15-day or 13-week studies. In the 2-year studies, C-cell adenomas/carcinomas of the thyroid gland showed a positive trend ($P = 0.03$) for male rats (control, 1/50; low dose, 1/49; high dose, 6/47). The incidence of C-cell neoplasms in the high dose was not significantly increased compared with the controls, and the occurrence of C-cell hyperplasia was not elevated (4/50; 3/49; 1/47). The incidence of alveolar/bronchiolar adenomas or carcinomas (combined) in male rats with a positive trend, and the incidence in the high dose group was greater than that in the controls (0/50; 3/50; 4/50). This marginal effect was not supported by an increase in epithelial hyperplasia (5/50; 5/50; 3/50). These marginal increases in male rats were not regarded as being related to the administration of 8-hydroxyquinoline.

In in vitro tests, 8-hydroxyquinoline did not induce either unscheduled DNA synthesis in rat hepatocytes or transformation of BALB/c-3T3 cells.

An audit of the experimental data for these carcinogenesis studies on 8-hydroxyquinoline was conducted. No data discrepancies were found that significantly influenced the final interpretations.

Under the conditions of these studies, there was *no evidence of carcinogenicity* for male and female F344/N rats or for male and female B6C3F$_1$ mice given 8-hydroxyquinoline in feed at concentrations of 1,500 or 3,000 ppm for 103 weeks.

Synonyms: 8-quinolinol; oxine; hydroxybenzopyridine

Report Date: April 1985

TR-277 Toxicology and Carcinogenesis Studies of Tremolite (CAS No. 14567-73-8) in F344/N Rats (Feed Studies)

A carcinogenesis bioassay of blocky (nonfibrous) tremolite was conducted with male and female F344/N rats. Tremolite was administered at a concentration of 1% in pelleted diet for the entire lifetime of the rats, starting with the dams of the study animals. The studies were started in 1978 and ended in 1981. Group sizes were 118 male and female controls and 250 male and female tremolite-exposed rats.

Litter size was not effected by the administration of tremolite to the dams. The offspring from mothers exposed to tremolite were the same size at birth as the controls but were slightly smaller at weaning and remained so throughout their life. Survival was similar in the exposed and control groups. No toxicity or increase in incidence of neoplasia was observed in the tremolite-exposed animals compared with the concurrent controls.

Conclusions: Under the conditions of these feed studies, nonfibrous tremolite was not overly toxic or carcinogenic for male or female F344/N rats, following lifetime ingestion of a diet containing 1% tremolite.

Report Date: March 1990

TR-278 Toxicology and Carcinogenesis Studies of 2,6-Xylidine (2,6-Dimethylaniline) (CAS No. 87-62-7) in Charles River CD Rats (Feed Studies)

2,6-Xylidine is a chemical intermediate used principally in the production of dyes. It is also a component of tobacco smoke, a degradation product of aniline-based pesticides, and a metabolite of certain drugs, particularly the xylide group of local anesthetics. The National Toxicology Program (NTP) sponsored single-administration, 2-week, and 13-week studies of 2,6-xylidine by gavage in F344/N rats. The U.S. Environmental Protection Agency (EPA) sponsored short-term gavage studies and 10-week range-finding studies in Charles River CD rats (a Sprague Dawley-derived strain). A carcinogenesis study of 2,6-xylidine was initiated by the EPA, which designed and monitored the study during the 2-year exposure period. The NTP then assumed responsibility for the study, conducting terminal kill, necropsy, histopathologic evaluation, data analysis, and report preparation.

Oral LD50 values of 1.2-1.3 g/kg were calculated for F344/N and Charles River CD rats administered single doses of 2,6-xylidine. Marginally toxic effects occurred in the hepatic, renal, and hematopoietic systems of dosed rats in the single-administration, 2-week, 10-week, and 13-week studies.

The 56 male and 56 female Charles River CD rats used in the 104-week carcinogenesis studies were the offspring of animals fed diets containing 0, 300, 1,000, or 3,000 ppm 2,6-xylidine before breeding, during pregnancy, and through the lactation period. The concentrations of 2,6-xylidine offered to animals in the 104-week studies were the same as those given to their parents.

During most of the 2-year studies, high dose male and female rats showed a reduction (greater than 10%) in body weight gain. Survival in the high dose male rats was significantly reduced ($P<0.001$) relative to that in controls. Survival also was reduced in the 1,000-ppm group. There was no significant relationship between concentration and mortality in female rats, but mortality was high for all groups of female rats during the second year of the study.

The epithelium of the nasal cavity was the primary site of compound-related neoplastic and nonneoplastic lesions. The incidences of both papillomas and carcinomas of the nasal cavity were significantly increased in high dose male and female rats. Carcinomas or adenocarcinomas (combined) occurred in 28/56 high dose males, 24/56 high dose females, and 1/56 mid dose females. Papillary adenomas occurred in 10/56 high dose males, 2/56 mid dose males, and 6/56 high dose females. None occurred in the other groups. The carcinomas were highly invasive and frequently destroyed the nasal turbinates and nasal septum. Metastasis to the brain was present in 5/56 male and 7/56 females high dose rats.

Malignant mesenchymal tumors were observed in the nasal cavity. Rhabdomyosarcomas occurred in two high dose male rats and two high dose female rats. These rare malignant tumors have not been previously reported at this site in Sprague Dawley rats. Malignant mixed tumors having features of adenocarcinomas and rhabdomyosarcomas were reported in one high dose male and one high dose female rat. One undifferentiated sarcoma was seen in a high dose female rat. The nonneoplastic lesions observed in the nasal cavity included acute inflammation, epithelial hyperplasia, and squamous metaplasia.

The incidences of subcutaneous tissue fibromas were increased in high dose male and female rats (male: control, 0/56; low dose, 1/56; mid dose, 2/56; high dose, 4/56; female: 0/56; 2/56; 1/56; 4/56) and were dose related. Subcutaneous fibrosarcomas were observed in three high dose females, one high dose male, one mid dose female, one low dose male, and one control female.

A significant dose-related increase occurred in the incidence of female rats with neoplastic nodules of the liver (0/56; 1/56; 2/56; 4/55). This increase was significant in the high dose group by the incidental tumor test.

Conclusions: Under the conditions of these 2-year feed studies, 2,6-xylidine was clearly carcinogenic for male and female Charles River CD rats, causing significant increases in the incidences of adenomas and carcinomas of the nasal cavity. A rhabdomyosarcoma, a rare tumor of the nasal cavity, was observed in dosed rats of each sex. In addition, the increased incidences of subcutaneous fibromas and fibrosarcomas in male and female rats and the increased incidence of neoplastic nodules of the liver in female rats may have been related to the administration of 2,6-xylidine.

Synonym: 2,6-Dimethylaniline

Report Date: January 1990

TR-279 Toxicology and Carcinogenesis Studies of Amosite Asbestos (CAS No. 12172-73-5) in F344/N Rats (Feed Studies)

The term "asbestos" has a commercial/industrial derivation limited to naturally occurring fibrous minerals of the serpentine or amphibole series. Chrysotile is the only type of asbestos in the serpentine series, whereas the amphibole series is represented by actinolite, amosite, anthophyllite, crocidolite, and tremolite. The essential characteristic of asbestos minerals is their fibrous nature.

Large portions of the population ingest asbestos through consumption of food and water. Asbestos or asbestos-like fibers may gain access to water supplies as a result of mining (Lake Superior), from the presence of natural serpentine or or amphibole deposits in watersheds (Seattle, WA, and San Francisco, CA) or, under certain conditions, through the use of asbestos-cement pipes for municipal water supplies. For the latter, erosion of the pipe with release of fibers is associated with the "aggressiveness" of the water, a term representing a mathematical expression of pH, alkalinity, and calcium content. The EPA estimated that 68.5% of water systems in the United States utilize water that is potentially capable of eroding asbestos-cement pipe.

Carcinogenesis studies of amosite asbestos alone or in combination with the intestinal carcinogen 1,2-dimethylhydrazine dihydrochloride (DMH) were conducted in male and female rats. Amosite asbestos was administered at a concentration of 1% in pelleted diet for the entire lifetime of the rats, starting with the dams of the study animals. One group of amosite asbestos-exposed rats (amosite preweaning gavage) also received chrysotile asbestos via gavage during lactation. Group sizes varied from 100 to 250. Litter size was the same, but the offspring from mothers exposed to amosite asbestos were smaller at weaning than those from nonexposed mothers and remained smaller throughout their life. The DMH was administered by gavage at a dose of 7.5 mg/kg for males and 15 mg/kg for females every 14 days, starting at 8 weeks of age, for a total of five doses. The administration of DMH did not affect body weight gain either in amosite-exposed or nonexposed animals.

The amosite-exposed rats showed enhanced survival compared with that of the nonexposed rats. DMH exposure reduced survival by approximately 1 year, although the survival of the amosite plus DMH groups

was slightly greater than that of the DMH group alone.

Significant increases in the incidences of C-cell carcinomas of the thyroid gland (untreated control, 11/117; amosite, 50/246, $P<0.05$; amosite preweaning gavage, 14/100) and of leukemia (38/117; 106/249, $P<0.05$; 49/100, $P<0.01$) in male rats were observed in amosite-exposed groups. However, the biologic significance of the C-cell carcinomas in relation to amosite asbestos exposure is discounted because of a lack of significance when C-cell adenomas and carcinomas were combined and because the positive effect was not observed in the amosite preweaning gavage group. The biologic significance of an increased incidence of leukemia is questionable because of a lack of statistical significance in the amosite group when evaluated by life table analysis and because no toxic lesions were observed in the target organs, i.e., gastrointestinal tract and mesothelium.

DMH caused a high incidence (62%-74%) of intestinal neoplasia in amosite-exposed and nonexposed groups. Neither an enchanced carcinogenic nor a protective effect was demonstrated by exposure to amosite asbestos.

Conclusions: Under the conditions of these feed studies, amosite asbestos was not overtly toxic, did not affect survival, and was not carcinogenic when ingested at a concentration of 1% in the diet by male or female F344/N rats. The cocarcinogenic studies using DMH were considered inadequate because of the high incidence of DMH-induced intestinal neoplasia in both the amosite asbestos-exposed and nonexposed groups.

Report Date: November 1990

Note: Amosite Asbestos was previously tested in Syrian Golden Hamsters administered in feed (See TR-249, reported 1983).

TR-280 Toxicology and Carcinogenesis Studies of Crocidolite Asbestos (CAS No. 12001-28-4) In F344/N Rats (Feed Studies)

Carcinogenesis studies of crocidolite asbestos were conducted with male and female F344/N rats. This form of asbestos was administered at a concentration of 1% in pelleted diet for the lifetime of the rats, starting with the dams of the study animals. The studies were started in January 1978 and ended in December 1980. Group sizes were 118 for male and female controls and 250 for male and female crocidolite asbestos-exposed rats.

The offspring from mothers exposed to crocidolite asbestos and the controls were similar in size at birth but were slightly smaller at weaning and remained so throughout their life. Feed consumption and survival were comparable in the exposed and control groups. No overt toxicity was observed in the crocidolite asbestos-exposed animals. There was an elevated ($P<0.05$) incidence of thyroid gland C-cell adenomas (control, 4/117, 3%, vs. exposed, 23/250, 9%) and of thyroid gland C-cell carcinomas (12/117, 10%, vs. 46/250, 18%) in crocidolite asbestos-exposed female rats relative to concurrent controls. Because these control incidences were low relative to control incidences observed in other contemporary studies at this laboratory (21% for thyroid gland C-cell tumors), this slight increase was not regarded as being biologically important.

The data, documents, and pathology materials from the lifetime studies of crocidolite asbestos have been audited. The audit findings show that the conduct of these studies is documented adequately and support the data and results presented in this Technical Report.

Conclusions: Under the conditions of these feed studies, crocidolite asbestos was not overtly toxic and did not cause a carcinogenic response when ingested at a concentration of 1% in the diet by male and female F344/N rats for their lifetime.

Report Date: December 1988

TR-281 Toxicology and Carcinogenesis Studies of HC Red No. 3 [2,((Amino-2-nitrophenyl)amino)ethanol] (CAS No. 2871-01-4) In F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of HC Red No. 3 (97% pure), a semipermanent hair dye, were conducted by administering the chemical in corn oil by gavage for 105 weeks to groups of 50 male and 50 female F344/N rats and for 104 weeks to groups of 50 male and 50 female B6C3F$_1$ mice. The dosage regimen used for rats was 0, 250, or 500 mg/kg per day and for mice, 0, 125, or 250 mg/kg per day. Doses were administered 5 days per week. In prior 13-week studies, these doses produced no signs of toxicity when administered 5 days per week.

In the 2-year studies, the administration of HC Red No. 3 did not affect body weight gains of male or female rats or mice. Body weight gains by all groups of female mice were reduced because of a reproductive tract infection. Survival of male and female rats and mice was not reduced by administration of HC Red No. 3. The survival of female mice, including vehicle controls, was reduced relative to historical survival rates due to a reproductive tract infection. The infection, accompanied by weight loss, high mortality, and suppurative inflammation of multiple organs, was found in 36/50 vehicle control, 32/50 low dose, and 29/50 high dose female mice. *Klebsiella pneumoniae* was isolated from infected tissues.

Pigmentation of various tissues in both rats and mice was a common observation in both the 13-week and the 2-year studies. The pigment was not identified but was presumed to be a derivative of HC Red No. 3. Very minimal nephropathy was found in dosed female rats, but its relationship to HC Red No. 3 is equivocal. Mild nephrosis was found in dosed female mice, but this effect may have been secondary to the infection of the genital tract.

There was an increase in the incidence of mammary gland fibroadenomas or cystadenomas in low dose female rats. The incidence of this lesion in high dose female rats

was not increased (vehicle control, 14/50, 28%; low dose, 25/50, 50%; high dose, 11/50, 22%). Largely because of the lack of a dose response, the increased incidence in the low dose females was not considered to be due to HC Red No. 3. No increased incidences of neoplasms were seen in male rats.

Transitional cell papillomas of the urinary bladder were detected in one high dose male rat, two low dose female rats, and one high dose female rat; none was observed in the vehicle controls. These uncommon neoplasms were found in animals that survived to the termination of the study and were not accompanied by other proliferative lesions.

The incidence of hepatocellular adenomas or carcinomas (combined) was increased in high dose male mice, whereas the incidence of these neoplasms in low dose male mice was significantly lower than that in the vehicle controls (25/50; 15/50; 35/50). Hepatocellular carcinomas in three vehicle control, one low dose, and five high dose male mice metastasized to the lung. The incidences of liver neoplasms in dosed female mice were not significantly different from those in the vehicle control group.

HC Red No. 3 was mutagenic in *Salmonella typhimurium* strains TA97, TA98, and TA100, but not in TA1535, in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9 when tested by the preincubational protocol.

An audit of the experimental data was conducted for these 2-year toxicology and carcinogenesis studies on HC Red No. 3. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies of HC Red No. 3, there was *no evidence of carcinogenicity* for male or female F344/N rats given 250 or 500 mg/kg per day. There was *equivocal evidence of carcinogenicity* for male $B6C3F_1$ mice as indicated by an increased incidence of hepatocellular adenomas or carcinomas (combined) in the 250 mg/kg dose group. Poor survival coupled with lack of significant findings rendered the study in female $B6C3F_1$ mice an *inadequate study of carcinogenicity*. Both sexes of both species may have been able to tolerate higher doses of HC Red No. 3. Therefore, the sensitivity of these studies for detecting carcinogenesis may have been limited.

Synonym: 2,((amino-2-nitrophenyl)amino)ethanol

Report Date: January 1986

TR-282 Toxicology and Carcinogenesis Studies of Chlorodibromomethane (CAS No. 124-48-1) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of chlorodibromomethane (greater than 98% pure), a trihalomethane formed after chlorination of water supplies, were conducted by administering this test chemical in corn oil gavage five times per week for 104 weeks to groups of 50 male and 50 female F344/N rats at 0,40, or 80 mg/kg per day and to groups of 50 male and 50 female $B6C3F_1$ mice for 105 weeks at doses of 0, 50, or 100 mg/kg per day. Survival of dosed male and female rats and female mice was comparable to that of the corresponding vehicle control groups. An overdose of chemical was given to low dose male and female mice at week 58; this overdose killed 35 male mice, whereas the female mice were apparently not affected. Because this mortality significantly reduced the number of survivors, the low dose male mouse group was considered to be inadequate for analysis of neoplasms. High dose male mice had lower survival than the vehicle controls (44/50 vs 29/50; $P<0.001$). At week 82, nine high dose male mice had died; the cause remains unknown. High dose male rats and dosed male and female mice had lower body weights compared with those of the vehicle controls.

Compound-related nonneoplastic lesions were found in the liver and kidney in male and female rats and in male mice in the 13-week studies at the highest dose (250 mg/kg). In the 2-year studies, compound-related toxicity was seen primarily in the livers of male and female rats (fatty metamorphosis and ground-glass cytoplasmic changes) and in the male mice (hepatocytomegaly, necrosis, fatty metamorphosis) and female mice (calcification and fatty metamorphosis). Toxicity was also seen in the kidneys (nephrosis) of male mice and female rats.

Administration of chlorodibromomethane significantly increased the incidence of hepatocellular adenomas (vehicle control, 2/50; low dose, 4/49; high dose, 11/50) and the combined incidences of hepatocellular adenomas or carcinomas (6/50; 10/49; 19/50) in high dose female mice. The incidence of hepatocellular carcinomas (vehicle control, 10/50; high dose, 19/50) was significantly increased in high dose male mice, although the combined incidence of hepatocellular adenomas or carcinomas (vehicle control, 23/50; high dose, 27/50) was marginally significant by the life table test but not by the incidental tumor test.

Negative trends in several common rodent tumors were found in dosed animals in the 2-year studies. These neoplasms included fibroadenomas of the mammary gland in female rats, endometrial stromal polyps of the uterus in female rats, and malignant lymphomas in male mice.

Chlorodibromomethane was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of Aroclor-induced male Sprague-Dawley rat or male Syrian hamster liver S9.

An audit of the experimental data was conducted for the 2-year toxicology and carcinogenesis studies of chlorodibromomethane. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these gavage studies, there was *no evidence of carcinogenicity* in male or female F344/N rats receiving chlorodibromomethane at doses of 40 or 80 mg/kg five times per week for 104 weeks. Fatty metamorphosis and ground-glass cytoplasmic changes of the liver in male and female F344/N rats were related

to administration of chlorodibromomethane. There was *equivocal evidence of carcinogenicity* for male $B6C3F_1$ mice; chlorodibromomethane caused an increased incidence of hepatocellular carcinomas, whereas the combined incidence of hepatocellular adenomas or carcinomas was only marginally increased. *Some evidence of carcinogenicity* was observed for female $B6C3F_1$ mice, since chlorodibromomethane caused an increased incidence of hepatocellular adenomas and an increased combined incidence of hepatocellular adenomas or carcinomas.

Synonym: Dibromochloromethane

Report Date: August 1985

TR-283 Pyridine (CAS: 110-86-1)

Study considered inadequate; no Technical Report will be issued.

TR-284 Toxicology and Carcinogenesis Studies of Diallylphthalate (CAS No. 131-17-9) in F344/N Rats (Gavage Studies)

Diallylphthalate is widely used as a crosslinking agent for unsaturated polyesters. Diallylphthalate or diallylphthalate polyester blends are used primarily as plasticizers and carriers for adding catalysts and pigments to polyesters and in molding, electrical parts, laminating compounds, and impregnation of metal castings. Rubber compounds, epoxy formulations, and polyurethane foams may also contain diallylphthalate. Precise figures are not currently available, although annual production of diallylphthalate in the United States is known to exceed 5,000 pounds, and an estimated 57,000 pounds were imported into the United States in 1982.

Toxicology and carcinogenesis studies of diallylphthalate (approximately 99% pure) were conducted by administering the test chemical in corn oil by gavage to groups of 50 male and 50 female F344/N rats at doses of 0 (vehicle control), 50, or 100 mg/kg 5 days per week for 103 weeks. The diallylphthalate doses used in the 2-year studies were chosen on the basis of 13-week studies, wherein doses of 200 or 400 mg/kg caused death, reductions in body weight gains, or periportal hepatocellular necrosis and fibrosis in both sexes.

Mean body weights and survival of male and female rats administered diallylphthalate were essentially the same as those of the vehicle controls throughout the 2-year studies, although hepatotoxicity was produced in both sexes by the 100 mg/kg dose. Based on the results of the prechronic studies and the effects on the liver in the 2-year studies, the doses used in the 2-year studies were considered to be adequate for carcinogenicity testing.

Male and female rats receiving the 100 mg/kg dose of diallylphthalate in the 2-year studies developed chronic liver diseases characterized by periportal fibrosis, periportal accumulation of pigment, and severe bile duct hyperplasia. Pigment accumulation also occurred at the 50 mg/kg dose in both sexes.

Diallylphthalate administration increased the occurrence of mononuclear cell leukemia in female rats ($P<0.05$ by trend tests), and the increase in the 100 mg/kg dose female rats was greater ($P\leq 0.05$) than in the vehicle controls by pairwise comparisons (vehicle control, 15/50, 30%; low dose, 15/43, 35%; high dose, 25/49, 51%). An increased occurrence of mononuclear cell leukemia was not observed in male rats receiving diallylphthalate.

A previous NTP carcinogenesis study (NTP TR 242) reported an increased incidence of lymphomas in male $B6C3F_1$ mice receiving diallylphthalate by gavage for 2 years at doses of 0, 150, or 300 mg/kg. This increase was considered to be equivocally related to diallylphthalate administration. The incidences of hyperplasia and inflammatory lesions of the forestomach were increased in a dose-related fashion in both sexes of mice in that study, and uncommon forestomach papillomas were observed in 0%, 2%, and 4% of both sexes of mice. Because of the numerical increase in forestomach papillomas, the concomitant presence of forestomach hyperplasia, and the rarity of forestomach papillomas in vehicle control (corn oil gavage) $B6C3F_1$ mice, the development of these proliferative lesions of the forestomach in mice may have been related to diallylphthalate administration. In the current study in rats, a squamous cell carcinoma was found in one high dose male rat.

Diallylphthalate was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without activation by a 9,000 x *g* supernatant fraction from the livers of Aroclor 1254-treated male Sprague-Dawley rats or Syrian hamsters. Diallylphthalate did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*.

An audit of the experimental data was conducted for these carcinogenicity studies on diallylphthalate. No data discrepancies were found that influenced the final interpretations.

Under the conditions of this study, the administration of diallylphthalate by gavage in corn oil to male and female F344/N rats for 2 years caused chronic liver disease characterized by periportal fibrosis and pigment accumulation and an increased severity of bile duct hyperplasia. The incidence of mononuclear cell leukemia was significantly increased in female rats receiving 100 mg/kg. Because of the variability in the incidence of this neoplasm in aged Fisher 344 rats and the difficulty in definitively diagnosing this lesion in Fisher 344 rats, this increase was considered to be *equivocal evidence of carcinogenicity* of diallylphthalate in female rats. There was *no evidence of carcinogenicity* in male rats.

Synonym: DAP

Report Date: August 1985

Note: Diallylphthalate was previously tested in $B6C3F_1$ mice by gavage (See TR-242, reported 1983).

TR-285 Toxicology and Carcinogenesis Studies of C.I. Basic Red 9 Monohydrochloride (Pararosaniline) (CAS No. 569-61-9) In F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

C.I. Basic Red 9 monohydrochloride is a triphenylmethane dye used for coloring textiles, leather, and paper and as a biological stain. Toxicology and carcinogenesis studies were conducted by administering the test chemical in feed to groups of 50 male and 50 female F344/N rats and B6C3F$_1$ mice for 103 weeks at concentrations of 0, 1,000, or 2,000 ppm for male rats and 0, 500, or 1,000 ppm for female rats and mice of each sex. The average daily doses of C.I. Basic Red 9 monohydrochloride were estimated to be 49 and 103 mg/kg for male rats, 28 and 59 for female rats, 196 and 379 mg/kg for male mice, and 149 and 407 mg/kg for female mice. Two lots of the test chemical were used in the 2-year studies with purities of 93% (water content approximately 9%) and 99%.

In rats, the thyroid gland and pituitary gland were identified as target sites in the 13-week studies. Therefore, 10 additional rats of each sex were added to the control and high dose groups in the 2-year studies to examine the effects on these organs after 1 year of exposure.

In the 1-year studies in rats, final mean body weights were slightly decreased in both sexes. The thyroid gland weight to body ratio of dosed males was 1.7 times that of the controls, and the concentration of serum thyroxin in male and female rats was significantly lower than that of the controls at week 52. Compound-related histopathologic effects included thyroid gland cysts in both sexes (1/10; 1/10) and thyroid gland follicular cell hyperplasia (1/10), adenomas (1/10), and carcinomas (1/10) and fatty metamorphosis of the liver (4/10, two of these with focal necrosis) in males; no effect was seen in the controls.

The doses selected for the 2-year studies were based on the results of the 13-week studies. The absence of toxicologic signs, histopathologic changes, significant body weight depressions, or mortality after 13 weeks of exposure to C.I. Basic Red 9 monohydrochloride suggested that these concentrations would not shorten survival. However, throughout the 2-year studies, mean body weights of high dose rats and dosed mice were lower than those of the controls, and significantly reduced survival relative to controls was observed for high dose rats of each sex ($P<0.001$), low dose male mice ($P<0.03$), and low dose and high dose female mice ($P<0.001$).

In the 2-year studies, several types of neoplastic lesions occurred with significantly increased incidences in dosed animals (see table page 12 of the Technical Report). High dose male rats had increased incidences of squamous cell carcinomas, trichoepitheliomas, and sebaceous adenomas of the skin. Greater incidences of follicular cell carcinomas and of follicular cell adenomas were found in the thyroid glands of high dose male rats than in controls, whereas in high dose female rats, the combined incidence of follicular cell adenomas or carcinomas was greater than that in controls. Dosed rats of each sex had increased incidences of subcutaneous fibromas, and high dose rats had increased incidences of Zymbal gland carcinomas. Hepatocellular carcinomas were the compound-related neoplasms common to both species; the incidences were increased in both high dose male rats and in dosed mice of each sex. Dosed female mice had an increased incidence of pheochromocytomas or malignant pheochromocytomas. In addition, marginally increased incidences of mammary gland tumors (23/50; 32/50; 32/50) in female rats, and malignant lymphomas (17/50; 24/50; 25/50) in female mice were observed.

C.I. Basic Red 9 monohydrochloride was mutagenic in strains TA98 and TA100 of Salmonella typhimurium by the preincubational protocol with or without metabolic activation. It was not mutagenic in strains TA1535 and TA1537 in this system with or without metabolic activation. It was mutagenic in the L5178Y/TK$^{+/-}$ mouse lymphoma assay with or without metabolic activation. C.I. Basic Red 9 monohydrochloride did not induce chromosomal aberrations in Chinese hamster ovary cells; it did induce sister-chromatid exchanges in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver S9. C.I. Basic Red 9 monohydrochloride also induced unscheduled DNA synthesis in F344 male rat hepatocytes in vitro.

An audit of the experimental data was conducted for these 2-year studies of C.I. Basic Red 9 monohydrochloride. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenicity* of C.I. Basic Red 9 monohydrochloride for male and female F344/N rats and for male and female B6C3F$_1$ mice. In male rats, C.I. Basic Red 9 monohydrochloride caused squamous cell carcinomas, trichoepitheliomas and sebaceous adenomas of the skin, subcutaneous fibromas, thyroid gland follicular cell adenomas or carcinomas (combined), and Zymbal gland carcinomas. In male mice, C.I. Basic Red 9 monohydrochloride caused hepatocellular carcinomas. In female mice, C.I. Basic Red 9 monohydrochloride caused hepatocellular carcinomas and adrenal gland pheochromocytomas or malignant pheochromocytomas (combined). Exposure to C.I. Basic Red 9 monohydrochloride also may have been related to increased incidences of mammary gland tumors in female rats and hematopoietic system tumors in female mice.

Synonyms: pararosaniline; benzeneamine 4-((4-aminophenyl)(4-imino-2,5-cyclohexadian-1-ylidene) methyl)-monohydrochloride; paramagenta

Report Date: January 1986

TR-286 Hamamelis Water (Witch Hazel) (CAS: 68916-39-2)

Study considered inadequate; no Technical Report will be issued.

TR-287 Toxicology and Carcinogenesis Studies of Dimethyl Hydrogen Phosphite (CAS No. 868-85-9) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Dimethyl hydrogen phosphite (DMHP) is used as an intermediate in the production of insecticides and herbicides, as an additive to lubricants, and as a stabilizer in oil and plaster and was considered for use as a chemical to stimulate the physical (but not the biologic) properties of anticholinesterase agents. Results of 13-week gavage studies in F344/N rats (0-400 mg DMHP/kg body weight) and in B6C3F$_1$ mice (0-1,500 mg DMHP/kg body weight) were used to identify short-term toxicity and to establish doses for the 2-year toxicology and carcinogenesis studies. In these studies, dimethyl hydrogen phosphite (greater than 97% pure) was administered for 103 weeks in corn oil by gavage to groups of 50 male F344/N rats and to groups of 50 male and female B6C3F$_1$ mice at doses of 0, 100, or 200 mg/kg and to groups of 50 female F344/N rats at doses of 0, 50, or 100 mg/kg.

In the 2-year studies, survival of high dose male rats and high dose male mice was lower ($P<0.05$) than that of the vehicle controls (male rats: vehicle control, 39/50; low dose, 29/50; high dose, 23/50; male mice: 42/50; 34/50; 32/50). At the end of the studies, mean body weights were lower than those of the corresponding vehicle controls for high dose male rats (-15%), for high dose female rats (-5%), and for high dose male mice (-5%).

Dimethyl hydrogen phosphite caused dose-related increases in nonneoplastic and neoplastic lesions of the lung in male and female rats. In high dose male rats, there were increased incidences of lung neoplasms, including squamous cell carcinomas (0/50; 0/50; 5/50), alveolar/bronchiolar adenomas (0/50; 0/50; 5/50), and alveolar/bronchiolar carcinomas (0/50; 1/50; 20/50). In high dose female rats, there was a marginal increase in the incidence of alveolar/bronchiolar carcinomas of the lung (0/50; 1/49; 3/50). Hyperplasia of the lung and chronic interstitial pneumonia were increased in dosed male rats and in high dose female rats.

Dimethyl hydrogen phosphite caused increases in forestomach lesions in male and female rats. In male rats, there was an increased incidence of forestomach neoplasms, including squamous cell papillomas (0/50; 1/50; 3/50) and squamous cell carcinomas (0/50; 0/50; 3/50). High dose male rats had increased incidences of hyperkeratosis and hyperplasia of the forestomach. In high dose female rats, the incidence of forestomach hyperplasia was increased. Neoplastic lesions of the forestomach (a squamous cell papilloma and a squamous cell carcinoma) were found in two high dose female rats.

Mineralization of the cerebellum was seen in high dose male rats (12/49) and in no other group. Focal calcification of the testis occurred at increased incidence in dosed male mice in the 2-year studies (2/50; 9/47; 24/50). Compound-related testicular atrophy was seen in male mice in the 13-week study.

Dimethyl hydrogen phosphite did not induce any neoplasms in male or female mice.

Dimethyl hydrogen phosphite was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. This chemical did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*.

An audit of the experimental data was conducted for these carcinogenic studies on dimethyl hydrogen phosphite. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these gavage studies, there was *clear evidence of carcinogenicity* in male rats receiving dimethyl hydrogen phosphite, as shown by increased incidences of alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, and squamous cell carcinomas of the lung and of neoplasms of the forestomach. There was *equivocal evidence of carcinogenicity* in female F344/N rats receiving dimethyl hydrogen phosphite, as shown by marginally increased incidences of alveolar/bronchiolar carcinomas of the lung and of neoplasms of the stomach. There was *no evidence of carcinogenicity* in male or female B6C3F$_1$ mice receiving dimethyl hydrogen phosphite at doses of 100 or 200 mg/kg for 103 weeks.

Synonyms: phosphonic acid, dimethyl ester (9CI); dimethyl phosphite; dimethyl phosphorus acid; methyl phosphonate; dimethyl phosphonate; dimethoxyphosphine oxide; TL 585; DMHP; phosphorous acid, dimethyl ester; dimethylphosphite; dimethylphosphonate; dimethylphosphorous acid; bis(hydroxymethyl) phosphine oxide

Report Date: November 1985

TR-288 Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No. 106-99-0) in B6C3F$_1$ Mice (Inhalation Studies)

1,3-Butadiene is used as an intermediate in the production of elastomers, polymers, and other chemicals. Of the 1,3-butadiene used in 1978, 44% was used to manufacture styrene-butadiene rubber (a substitute for natural rubber, produced by copolymerization of 1,3-butadiene with styrene), and 19% was used to produce polybutane elastomer (a substance that increases resistance of tire products to wear, heat degradation, and blowouts). Chloroprene monomer, derived from 1,3-butadiene, is used exclusively to manufacture neoprene elastomers for non-

tire and latex applications. Commercial nitrile rubber, used largely in rubber hoses, seals, and gaskets for automobiles, is a copolymer of 1,3-butadiene and acrylonitrile. Acrylonitrile-butadiene-styrene resins, usually containing 20%-30% 1,3-butadiene by weight, are used to make parts for automobiles and appliances. Other polymer uses include specialty polybutadiene polymers, thermoplastic elastomers, nitrile barrier resins, and K resins®. 1,3-Butadiene is used as an intermediate in the production of a variety of industrial chemicals, including two fungicides, captan and captofol. It is approved by the U.S. Food and Drug Administration for use in the production of adhesives used in articles for packaging, transporting, or holding food; in components of paper and paperboard that are in contact with dry food; and as a modifier in the production of semigrid and rigid vinyl chloride plastic food-contact articles. No information was located on the levels of monomer or on its elution rate from any of the commercially available polymers. It is not known if unreacted 1,3-butadiene migrated from packaging materials.

Male and female B6C3F$_1$ mice were exposed to air containing 1,3-butadiene (greater than 99% pure) at concentrations of 0-8,000 ppm in 15-day and 14-week inhalation studies. In the 15-day studies, survival was unaffected by dose, and no pathologic effects were observed; slight decreases in mean body weight occurred at the high concentrations. In the 14-week studies, mean body weight gain decreased with dose, and survival in the 5,000-ppm and 8,000-ppm groups of males was markedly reduced; no other compound-related effects were reported.

Inhalation carcinogenesis studies of 1,3-butadiene were conducted by exposing groups of 50 male and female B6C3F$_1$ mice 6 hours per day for 5 days per week to air containing the test chemical at concentrations of 0 (chamber controls), 625, or 1,250 ppm. These studies were planned for 103-week exposures but were terminated at week 60 for male mice and week 61 for female mice because of the rapidly declining survival, primarily due to neoplasia. Body weights were not affected by 1,3-butadiene.

Significantly increased incidences of neoplasms at multiple sites were observed in mice exposed to 1,3-butadiene. Hemangiosarcomas of the heart occurred at increased incidences in exposed males and females (male: control, 0/50; low dose, 16/49; high dose, 7/49; female: 0/50; 11/48; 18/49). Hemangiosarcomas were also observed in the peritoneal cavity (one high dose male), subcutaneous tissue (two low dose females), and liver (one high dose female).

Malignant lymphomas, diagnosed as early as week 20, were observed at increased incidences in exposed male and female mice (male: 0/50; 23/50; 29/50; female: 1/50; 10/49; 10/49).

Alveolar/bronchiolar adenomas and alveolar/bronchiolar (both separately and combined) occurred at increased incidences in exposed male and female mice (combined incidences – male: 2/50; 14/49; 15/49; female: 3/49; 12/48; 23/49).

Epithelial hyperplasia of the forestomach occurred at increased incidences in dosed mice (male: 0/49; 5/40; 7/44; female: 0/49; 5/42; 9/49). Papillomas of the forestomach occurred in low dose male and in low dose and high dose female mice (male: 0/49; 5/40; 0/44; female: 0/49; 4/42; 10/49). Squamous cell carcinomas of the forestomach were observed in dosed mice (male: 0/49, 2/40, 1/44; female: 0/49, 1/42, 1/49).

Acinar cell carcinomas of the mammary gland were observed at an increased incidence in high dose female mice (0/50; 2/49; 6/49); adenosquamous carcinomas were found in four low dose females. The incidences of granulosa cell tumors of the ovary were increased in dosed females (0/49; 6/45; 12/48). A granulosa cell carcinoma was observed in another high dose female. Gliomas were observed in two 68- to 69-week-old low dose and one high dose male mice; brain tumors are uncommon even in 2-year old mice.

Liver necrosis occurred at increased incidences in dosed male and low dose female mice (male; 1/50, 8/49, 8/49; female: 6/50, 15/47, 6/49). Hepatocellular adenomas or carcinomas (combined) were observed at an increased incidence in high dose female mice (0/50, 2/47 5/49).

No neoplastic lesions of the nasal cavity were observed at any dose level. The following nonneoplastic lesions of the nasal cavity occurred in mice exposed at 1,250 ppm: chronic inflammation (male, 35/50; female, 2/49); fibrosis (male, 33/50; female, 2/49); cartilaginous metaplasia (male, 16/50; female, 1/49); osseous metaplasia (male, 11/50; female, 2/49); and atrophy of the sensory epithelium (male, 32/50). No nonneoplastic lesions of the nasal cavity were found in the controls. The incidence of testicular atrophy (0/50, 19/49, 11/48) or ovarian atrophy (2/49, 40/45, 40/48) was increased in exposed male or female mice.

An audit of the experimental data from these studies on 1,3-butadiene was conducted by the National Toxicology Program. No data discrepancies were found that influenced the final interpretation of these experiments.

Under the conditions of these studies, there was *clear evidence of carcinogenicity* for 1,3-butadiene in male and female B6C3F$_1$ mice, as shown by increased incidences and early induction of hemangiosarcomas of the heart, malignant lymphomas, alveolar/bronchiolar adenomas and carcinomas, and papillomas of the stomach in males and females; and of acinar cell carcinomas of the mammary gland, granulosa cell tumors of the ovary, and hepatocellular adenomas and adenomas or carcinomas (combined) in females. 1,3-Butadiene was associated with nonneoplastic lesions in the respiratory epithelium, liver necrosis, and testicular or ovarian atrophy.

Synonyms: butadiene; biethylene; bivinyl; divinyl; erythrene; vinylethylene; pyrrolylene

Report Date: August 1984

TR-289 Toxicology and Carcinogenesis Studies of Benzene (CAS No. 71-43-2) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Benzene ranks 16th in production volume for chemicals produced in the United States, with approximately 9.9 billion pounds being produced in 1984, 9.1 billion pounds in 1983, and 7.8 billion pounds in 1982. This simplest aromatic chemical in used in the synthesis of styrene (polystyrene plastics and synthetic rubber), phenol (phenolic resins), cyclohexane (nylon), aniline, maleic anhydride (polyester resins), alkylbenzenes (detergents), chlorobenzenes, and other products used in the production of drugs, dyes, insecticides, and plastics. Benzene, along with other light, high-octane aromatic hydrocarbons, such as toluene and xylenes, is a component of motor gasoline. Benzene is also used as a solvent, but for most applications, it has been replaced by less hazardous solvents.

During the 17-week studies, groups of 10 or 15 male and female F344/N rats and B6C3F$_1$ mice were gavaged 5 days per week with benzene in corn oil (5 ml/kg) at doses of 0 to 600 mg/kg. No benzene-related deaths occurred; in rats that received benzene, final mean body weights were 14%-22% lower compared with vehicle controls and in mice, slight dose-related reductions were observed (less than 10% differences). Doses for the 2-year studies were selected based on clinical observations (tremors in higher dosed mice), on clinical pathologic findings (lymphoid depletion in rats and leukopenia in mice), and on body weight effects.

Two-year toxicology and carcinogenesis studies of benzene (greater than 99.7% pure) were conducted in groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex and for each dose. Doses of 0, 50, 100, or 200 mg/kg body weight benzene in corn oil (5 ml/kg) were administered by gavage to male rats, 5 days per week, for 103 weeks. Doses of 0, 25, 50, or 100 mg/kg benzene in corn oil were administered by gavage to female rats and to male and female mice for 103 weeks. Ten additional animals in each of the 16 groups were killed at 12 months and necropsies were performed. Hematologic profiles were performed at 3-month intervals. These studies were designed and conducted because of large production volume and potential human exposure, because of the epidemiologic association with leukemia, and because previous experiments were considered inadequate or inconclusive for determining potential carcinogenicity in laboratory animals.

In the 2-year studies, mean body weights of the 200 mg/kg male rats (-23%) and the 100 mg/kg mice (-14% to -19%) were lower than those of the vehicle controls, and survival of dosed groups decreased with increasing dose (rats—male: vehicle control, 32/50; low dose, 29/50; mid dose, 25/50; high dose, 16/50; female: 46/50; 38/50; 34/50; 25/50; mice—male: 28/50; 23/50; 18/50; 7/50; female: 30/50; 26/50; 24/50; 18/50). At week 92 for rats and week 91 for mice, survival was greater than 60% in all groups; most of the dosed animals that died before week 103 had neoplasia.

Compound-related nonneoplastic or neoplastic effects on the hematopoietic system, Zymbal gland, forestomach, and adrenal gland were found both for rats and mice. Further, the oral cavity was affected in rats, and the lung, liver, harderian gland, preputial gland, ovary, and mammary gland were affected in mice. Significantly increased ($P<0.05$) incidences of neoplasms were observed at multiple sites for male and female rats and for male and female mice. Primary neoplasms observed in rats and mice are summarized in Table 1 (see page 12 of the Technical Report).

Hematologic data from vehicle control and dosed rats and mice were obtained at 3-month intervals from 0 to 24 months. Reliably identifiable hematologic effects were limited to lymphocytopenia and associated leukocytopenia in benzene-dosed rats and mice. These effects were seen from 3 to 18 months in dosed male rats and in dosed male mice; a similar but less pronounced response was observed in dosed female rats during this same time period. The effect in female mice was limited to 12-18 months. The technical quality of certain of these data was questionable; thus, more detailed analyses (e.g., investigation of the association between hematologic and pathologic changes) are deemed inappropriate for these data. Benzene increased the frequency of micronucleated normchromatic peripheral erythrocytes in male and female mice (rats were not examined); males were more sensitive than females.

The hematopoietic system of rats and mice of each sex was affected by benzene in the 2-year studies. The incidences of malignant lymphomas in all dosed groups of mice were greater than those in the vehicle controls (male: 4/49; 9/48; 9/50; 15/49; female: 15/49; 24/45; 24/50; 20/49). Lymphoid depletion of the splenic follicles (rats) and thymus (male rats) was observed at increased incidences. Bone marrow hematopoietic hyperplasia was observed at increased incidences in dosed mice of each sex (male: 0/49; 11/48; 10/50; 25/49; female: 3/49; 14/45; 8/50; 13/49).

The incidences of Zymbal gland carcinomas in mid and high dose male rats and in dosed female rats were greater than those in the vehicle controls (male: 2/32; 6/46; 10/42; 17/42; female: 0/45; 5/40; 5/44; 14/46). The incidences of Zymbal gland carcinomas in mid and high dose male mice and in high dose female mice were greater than those in the vehicle controls (male: 0/43; 1/34; 4/40; 21/39; female: 0/43; 0/32; 1/37; 3/31). In mid and high dose male mice and in high dose female mice, the incidences of epithelial hyperplasia of the Zymbal gland were also increased (male: 0/43; 3/34; 12/40; 10/39; female: 1/43; 1/32; 2/37; 6/31).

Hyperplasia of the adrenal capsule was observed at increased incidences in dosed mice of each sex (male: 2/47; 32/48; 14/49; 4/46; female: 5/49; 19/44; 34/50; 30/48). The incidence of pheochromocytomas in mid dose male mice was greater than that in the vehicle controls (male: 1/47; 1/48; 7/49; 1/46), whereas the incidences in dosed female mice were lower than that in the vehicle controls (female: 6/49; 1/44; 1/50; 1/48). Hyperplasia of the zona fasciculata of the adrenal cortex was observed at

increased incidences in low dose rats of each sex (male: 0/50; 13/49; 0/48; 2/49; female: 0/50; 17/50; 0/47; 0/49).

Benzene was associated with increased incidences of neoplasms of the skin and oral cavity of rats. The incidences of squamous cell papillomas and squamous cell carcinomas of the skin in high dose male rats were greater than those in the vehicle controls (squamous cell papilloma: 0/50; 2/50; 1/50; 5/50; squamous cell carcinoma: 0/50; 5/50; 3/50; 8/50). Increased incidences of uncommon squamous cell papillomas or squamous cell carcinomas (combined) of the oral cavity were observed in dosed male and female rats (male: 1/50; 9/50; 16/50; 19/50; female: 1/50; 5/50; 12/50; 9/50). Incidences of squamous cell papillomas or carcinomas (combined) (male: 2/45; 2/42; 3/44; 5/38; female: 1/42; 3/40; 6/45; 5/42), hyperkeratosis, and epithelial hyperplasia of the forestomach were increased in some dosed groups of male and female mice; incidences of hyperkeratosis and acanthosis were increased in high dose male rats.

Compound-related effects in the lung, harderian gland, preputial gland, ovary, mammary gland, and liver were seen in mice but not in rats. Administration of benzene was associated with increased incidences of alveolar epithelial hyperplasia in mid and high dose mice (male: 2/49; 3/48; 7/50; 10/49; female: 1/49; 1/42; 9/50; 6/49). Increased incidences of alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined) were observed in high dose male mice (carcinomas: 5/49; 11/48; 12/50; 14/49; adenomas or carcinomas: 10/49; 16/48; 19/50; 21/49). Alveolar/bronchiolar adenomas were seen at increased incidences in high dose female mice (4/49; 2/42; 5/50; 9/49), as were alveolar/bronchiolar carcinomas (0/49; 3/42; 6/50; 6/49) and alveolar/bronchiolar adenomas or carcinomas combined (4/49; 5/42; 10/50; 13/49) in mid and high dose female mice.

The incidences of focal or diffuse hyperplasia of the harderian gland were increased in dosed mice of each sex (male: 0/49; 5/46; 11/49; 7/48; female: 6/48; 10/44; 11/50; 10/47). The incidences of harderian gland adenomas (0/49; 9/46; 13/49; 11/48) in dosed male mice were greater than that in the vehicle controls. A marginal increase in the incidence of adenomas or carcinomas (combined) of the harderian gland was seen in high dose female mice (5/48; 6/44; 10/50; 10/47).

The administration of benzene to male mice was associated with increased incidences of hyperplasia (1/21; 18/28; 9/29; 1/35) and squamous cell carcinomas (0/21; 3/28; 18/29; 28/35) of the preputial gland. Increased incidences of mammary gland carcinomas were found in mid dose and high dose female mice (0/49; 2/45; 5/50; 10/49) and carcinosarcomas in high dose female mice (0/49; 0/45; 1/50; 4/49).

Increased incidences of various uncommon neoplastic and nonneoplastic lesions of the ovary (papillary cystadenoma, luteoma, granulosa cell tumor, tubular adenoma, benign mixed tumor, epithelial hyperplasia, and senile atrophy) were associated with the administration of benzene to female mice. In mid and high dose female mice, the incidences of granulosa cell tumors (1/47; 1/44; 6/49; 7/48) and benign mixed tumors (0/47; 1/44; 12/49; 7/48) were greater than those in the vehicle controls.

Increased incidences of hepatocellular adenomas were observed in low dose female mice (1/49; 8/44; 5/50; 4/49) and hepatocellular adenomas or carcinomas (combined) in low dose and mid dose female mice (4/49; 12/44; 13/50; 7/49).

An audit of the experimental data was conducted for these 2-year carcinogenesis studies on benzene. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenicity* of benzene for male F344/N rats, for female F344/N rats, for male $B6C3F_1$ mice, and for female $B6C3F_1$ mice. For male rats, benzene caused increased incidences of Zymbal gland carcinomas, squamous cell papillomas and squamous cell carcinomas of the oral cavity, and squamous cell papillomas and squamous cell carcinomas of the skin. For female rats, benzene caused increased incidences of Zymbal gland carcinomas and squamous cell papillomas and squamous cell carcinomas of the oral cavity. For male mice, benzene caused increased incidences of Zymbal gland squamous cell carcinomas, malignant lymphomas, alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined), harderian gland adenomas, and squamous cell carcinomas of the preputial gland. For female mice, benzene caused increased incidences of malignant lymphomas, ovarian granulosa cell tumors, ovarian benign mixed tumors, carcinomas and carcinosarcomas of the mammary gland, alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, and Zymbal gland squamous cell carcinomas. Dose-related lymphocytopenia was observed for male and female F344/N rats and male and female $B6C3F_1$ mice.

Synonyms: benzol, cyclohexatriene, pyrobenzol

Report Date: April 1986

TR-290 Castor Oil (CAS: 8001-79-4)

Study considered inadequate; no Technical Report will be issued.

TR-291 Toxicology and Carcinogenesis Studies of Isophorone (CAS No. 78-59-1) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of isophorone (greater than 94% pure), a widely used solvent and chemical intermediate, were conducted by administering 0, 250, or 500 mg isophorone/kg body weight per day by gavage in corn oil to groups of 50 F344/N rats and 50 $B6C3F_1$ mice of each sex, 5 days per week for 103 weeks.

Doses selected for the 2-year studies were based on the 16-day studies in which rats and mice of each sex received doses of 0-2,000 mg/kg per day and on 13-week studies in which rats and mice of each sex received doses ranging from 0 to 1,000 mg/kg per day by gavage in corn oil. No chemically related gross or histopathologic effects were observed in the 16-day or 13-week studies, but 1/5 high dose male rats, 4/5 high dose female rats, and all high dose male and female mice died during the 16-day studies. During the 13-week studies, 1/10 high dose female rats and 3/10 high dose female mice died. The high dose for the 2-year studies was set at 500 mg/kg per day for each sex of rats and mice, based mainly on the deaths in the 13-week studies.

Throughout the 2-year study, the mean body weights of the high dose male rats averaged 5% lower than those of the vehicle controls. During the second year, the mean body weights of the female high dose rats averaged 8% lower than those of the vehicle controls, and the high dose female mice averaged 5% lower. The survival of high dose male rats was significantly lower than that of the vehicle controls after week 96 (final survival: vehicle control, 33/50; low dose, 33/50; high dose, 14/50). The survival of dosed female rats was poor (30/50; 23/50; 20/50), due in part to 20 gavage-related accidental deaths of dosed animals. The survival of male mice was also low (16/50; 16/50; 19/50), but there was a significant trend toward increased survival of dosed female mice relative to that of the vehicle controls (26/50; 35/50; 34/50).

Dosed male rats showed a variety of proliferative lesions of the kidney (tubular cell hyperplasia: 0/50; 1/50; 4/50; tubular cell adenoma: 0/50; 0/50; 2/50; tubular cell adenocarcinoma: 0/50; 3/50; 1/50; epithelial hyperplasia of the renal pelvis: 0/50; 5/50; 5/50). Dosed male rats also exhibited increased mineralization of the medullary collecting ducts (1/50; 31/50; 20/50), and low dose male rats showed a more severe nephropathy than is commonly seen in aging F344/N rats. Carcinomas of the preputial gland were increased in high dose male rats (0/50; 5/50; 5/50). With the exception of a moderate increase in nephropathy (21/50; 39/50; 32/50), female rats did not show chemically related increased incidences of neoplastic or nonneoplastic lesions.

In high dose male mice, isophorone exposure was associated with increased incidences of hepatocellular adenomas and carcinomas (18/48; 18/50; 29/50) and of mesenchymal tumors of the integumentary system (fibroma, fibrosarcoma, neurofibrosarcoma, or sarcoma: 6/48; 8/50; 14/50). An increased incidence of lymphomas or leukemias was noted in low dose male mice (8/48; 18/50; 5/50). Coagulative necrosis (3/48; 10/50; 11/50) and hepatocytomegaly (23/48; 39/50; 37/50) were observed more frequently in the livers of dosed male mice than in vehicle controls. No compound-related neoplastic or nonneoplastic lesions associated with isophorone exposure were seen in female mice.

Isophorone was not mutagenic in strains TA100, TA1535, TA1537, or TA98 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9. Isophorone was weakly mutagenic in the mouse L5178Y/$TK^{+/-}$ assay in the absence of S9; it was not tested in the presence of S9. Isophorone induced sister-chromatid exchanges in the absence of S9 in Chinese hamster ovary cells; it did not induce sister-chromatid exchanges in the presence of Aroclor 1254-induced male rat liver S9, and it did not induce chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of S9.

An audit of the experimental data was conducted for the 2-year toxicology and carcinogenesis studies of isophorone. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenicity* of isophorone in male F344/N rats as shown by the occurrence of renal tubular cell adenomas and adenocarcinomas in animals given 250 or 500 mg/kg per day; carcinomas of the preputial gland were also observed at increased incidence in male rats given 500 mg/kg. There was *no evidence of carcinogenicity* in female F344/N rats given 250 or 500 mg/kg per day. For male $B6C3F_1$ mice, there was *equivocal evidence of carcinogenicity* of isophorone as shown by an increased incidence of hepatocellular adenomas or carcinomas (combined) and of mesenchymal tumors in the integumentary system in animals given 500 mg/kg per day and by an increase in malignant lymphomas in animals given 250 mg/kg per day. There was *no evidence of carcinogenicity* of isophorone in female $B6C3F_1$ mice given 250 or 500 mg/kg per day.

Synonym: 3,5,5-trimethyl-2-cyclohexen-1-one

Report Date: January 1986

TR-292 Trichlorfon (CAS: 52-68-6)

Study considered inadequate; no Technical Report will be issued.

TR-293 Toxicology and Carcinogenesis Studies of HC Blue No. 2 [2,2'-((4-((2-Hydroxyethyl)amino)-3-nitrophenyl) imino)bis(ethanol)] (CAS No. 33229-34-4) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Toxicology and carcinogenesis studies of HC Blue No. 2 (approximately 98% pure), a semipermanent hair dye, were conducted by administering the test chemical in feed for 103 weeks to groups of 50 F344/N rats of each sex and for 104 weeks to groups of 50 $B6C3F_1$ mice of each sex. The dietary concentrations used were 0, 5,000, or 10,000 ppm for male rats and male mice and 0, 10,000, or 20,000 ppm for female rats and female mice. These concentrations were selected on the basis of results from

single-administration gavage and 14-day and 13-week feed studies. For the 2-year studies, the average daily doses were approximately 195 and 390 mg/kg in male rats, 465 and 1,000 mg/kg in female rats, 1,320 and 2,240 mg/kg in male mice, and 2,330 and 5,600 mg/kg in female mice.

The survival of high dose male rats and male mice was better than that for controls, and the survival of dosed female rats was comparable to that of the controls. The survival of high dose female mice was reduced ($P<0.05$) relative to that of controls (control, 35/50; low dose, 27/50; high dose 19/50); this reduced survival was attributed to a reproductive tract infection. Final mean body weights relative to those of controls were depressed less than 10% in dosed male rats, whereas depressions of 13% and 22% were observed in the low dose and high dose groups of female rats. Final mean body weights for dosed male mice were within 5% of control values, but final mean body weights for dosed females were 15% (low dose) and 22% (high dose) lower than that of controls.

A dose-related increase in the incidence of hyperostosis of the skull was detected in rats (male, 5/50, 8/50, 25/49; female, 2/50, 19/50, 49/50) and in 1/49 high dose male and 4/50 high dose female mice. Mixed mesenchymal neoplasms of the kidney were detected in 2/50 high dose female rats; none was observed in any other group of female or male rats. This tumor is considered uncommon and has not been found in 1,863 historical control female F344/N rats. A negative trend in fibroadenomas of the mammary gland was seen in female rats (20/50, 10/50, 4/50).

A marginal ($P=0.05$) positive trend occurred in the incidence of lymphomas in male mice (1/50; 5/48; 8/49); the incidences in the dosed groups were not significantly greater than that in the controls when survival differences were taken into account.

HC Blue No. 2 was mutagenic for strains TA97 and TA98 but not for strains TA100 or TA1535 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. HC Blue No. 2 was mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay in the presence of Aroclor 1254-induced male F344/N rat liver S9.

An audit of the experimental data was conducted for these carcinogenic studies on HC Blue No. 2. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these studies, there was *no evidence of carcinogenicity* in male and female F344/N rats or in male and female B6C3F$_1$ mice receiving HC Blue No. 2 in the diet at concentrations of 0.5% and 1.0% for males and 1.0% and 2.0% for females for 2 years. HC Blue No. 2 administration caused a dose-related increase in the incidence of hyperostosis of the skull in male and female rats.

Synonym: 2,2'-((4-((-hydroxyethyl)amino)-3-nitrophenyl)imino)bis(ethanol)

Report Date: August 1985

TR-294 Toxicology and Carcinogenesis Studies of Chlorinated Trisodium Phosphate (CAS No. 56802-99-4)* in B6C3F$_1$ Mice (Gavage Studies)

Two-year toxicology and carcinogenesis studies of chlorinated trisodium phosphate, an inclusion complex of trisodium phosphate and sodium hypochlorite used in various cleaning compounds, were conducted by administering 0, 500, or 1,000 mg/kg (dose volume: 10 ml/kg) of the chemical in water by gavage, 5 days per week for 103 weeks, to groups of 50 male and 50 female B6C3F$_1$ mice. Groups of mice receiving 250 mg/kg were included in these studies but were removed after 6 months because of a lack of toxicity in the 500 and 1,000 mg/kg groups. Two-year studies were begun in male and female F344/N rats at doses of 0, 500, 1,000, or 2,000 mg/kg of chlorinated trisodium phosphate in water by gavage (10 ml/kg). The 2,000 mg/kg groups were killed at 15 weeks because of poor survival, and the other groups were killed at 35 weeks because of toxicity in the 1,000 mg/kg group. The doses selected for the 2-year studies were based on the general lack of adverse effects seen in the 14-day and 13-week studies in which rats received 0-1,000 mg/kg and mice received 0-2,000 mg/kg by gavage in water.

No compound-related histopathologic effects were observed in the 14-day or the 13-week studies in mice. In the 2-year studies, survival and mean body weights of dosed and vehicle control male mice groups were comparable (survival – vehicle control, 39/50; low dose, 35/50; high dose, 32/50). Survival of the dosed female mice was lower than that of the vehicle controls (30/50; 16/50; 21/50), although at week 80 survival of female mice was 42/50, 39/50, and 36/50. The mean body weights of the high dose female mice were lower than those of the vehicle control mice, primarily after week 32; final body weights were 11% lower in the high dose group compared with that in the vehicle controls. The lower survival and mean body weights of the dosed female mice may have been due to a greater incidence of uterine/ovarian infections in these mice rather than to a direct toxic effect of chlorinated trisodium phosphate. Nine of 20 vehicle control, 20/34 low dose, and 21/29 high dose female mice that died before the end of the studies had such infections. This reduced survival decreased the sensitivity of the study of female mice for detecting the presence or absence of carcinogenic effects.

At no site was the incidence of neoplasms considered to be related to the administration of chlorinated trisodium phosphate. minimal necrosis and fatty changes were observed in the livers of male mice. Kidneys in male mice were characterized by small, multifocal areas of mineralization, primarily in the cortex but not at the corticomedullary junction or in the tubes of the medulla. Neither effect was considered compound related. Five different types of ovarian neoplasms were found in six dosed female mice; because these lesions were from tissues of different embryonic origin, they were considered unrelated to administration of chlorinated trisodium phosphate.

Chlorinated trisodium phosphate was weakly mutagenic in strain TA1535 of *Salmonella typhimurium* in the presence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9. This compound was not mutagenic in strains TA97, TA98, or TA100.

An audit of the experimental data was conducted for these 2-year studies of chlorinated trisodium phosphate. No data discrepancies were found that influenced the final interpretation of these experiments.

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenicity* for either male or female $B6C3F_1$ mice given chlorinated trisodium phosphate by gavage in water for 103 weeks at doses of 500 or 1,000 mg. Survival of dosed female mice was 78% and 72% after 80 weeks and 32% and 42% at the termination of the study. The studies in male and female F344/N rats were considered to be *inadequate studies of carcinogenicity* because the experiments were terminated at 35 weeks due to poor survival.

Synonym: sodium hypochlorite phosphate

Report Date: December 1986

*The CAS Number (56082-99-4) indicated on the cover of the technical report is in error; the correct CAS number is indicated above.

TR-295 Toxicology and Carcinogenesis Studies of Chrysotile Asbestos (CAS No. 12001-29-5) in F344/N Rats (Feed Studies)

Lifetime toxicology and carcinogenesis studies of short-range (SR) and intermediate-range (IR) fiber length chrysotile asbestos were conducted in groups of 88-250 male and female F344/N rats. Both forms of asbestos were administered at a concentration of 1% in pelleted diet for the lifetime of the rats, starting with the dams of the test animals. Subgroups of 100 male and female IR chrysotile-exposed rats also received 0.47 mg/g IR chrysotile asbestos in water by gavage during lactation (preweaning [PW]). At 9 weeks of age, additional subgroups (125-175) of control and IR chrysotile-exposed rats received 7.5 mg/kg (male) or 15 mg/kg (female) 1,2-dimethylhydrazine dihydrochloride (DMH) by gavage every other week for a total of five doses. When the survival of either the control or test group reached 10%, both groups were killed.

Neither type of fiber affected fertility or litter size. The offspring from mothers exposed to SR chrysotile were similar in body weight to the controls at birth but were slightly smaller (13%) at weaning and remained so throughout their lifetimes. Feed consumption and survival were comparable among the SR and IR chrysotile asbestos groups and controls. The DMH-exposed groups showed decreased survival due primarily to the development of lethal neoplasms.

The administration of SR chrysotile for the lifetime of exposed male and female rats did not cause any overt toxicity. In addition, no neoplastic or nonneoplastic disease was associated with SR chrysotile exposure.

Male and female rats exposed to IR chrysotile asbestos did not show any adverse clinical signs. Benign epithelial neoplasms (adenomatous polyps) were observed in the large intestine of IR chrysotile asbestos male rats (9/250, 3.6%). Although not statistically significant ($P = 0.08$) compared with concurrent controls (0/85), the incidence of these neoplasms was highly significant ($P = 0.003$) when compared with the incidence of epithelial neoplasms (benign and malignant combined) of the large intestine in the pooled male control groups of all the NTP oral asbestos lifetime studies (3/524, 0.6%). The biologic importance of this finding was supported by the observation of lesions of similar morphology in the small intestine or glandular stomach of four additional IR chrysotile male rats and by a low incidence (2/100, 2.0%) of adenomatous polyps in the large intestine of male rats in the IR/PW group.

A significant ($P<0.05$) increase in keratoacanthomas of the skin was observed in male IR (19/250, 7.6%) and IR/PW (8/100, 8.0%) chrysotile-exposed rats compared with the concurrent controls (1/88, 1.1%). The biologic importance of this observation was discounted because the incidence in these groups did not greatly exceed the rate observed in the combined male control groups from all the other NTP oral asbestos studies (19/441, 4.3%). An apparent increase in the incidence of clitoral gland neoplasms in female IR (18/250, 7.2%) and IR/PW (4/100, 4.0%) chrysotile-exposed rats compared with that in the concurrent controls (1/88, 1.1%) was also discounted because of a lack of statistical significance when compared with the pooled female control groups from the other NTP oral asbestos studies (21/441, 4.8%).

Rats exposed to DMH and DMH plus IR chrysotile asbestos exhibited neoplasia in those organs known to be targets for DMH (gastrointestinal tract, Zymbal gland, liver, and kidney). There was a significant difference ($P<0.05$) in the incidence of DMH-induced mixed-cell tumors of the kidney between the DMH alone (13/125, 10%) and DMH plus IR chrysotile asbestos (34/175, 19%) female groups. An increased incidence of thyroid follicular cell tumors was observed in DMH plus IR chrysotile male rats (28/175, 16.0%) compared with the DMH alone group (9/124, 7.3%). The biologic importance of both observations is questionable, since neither organ represents a primary target organ for asbestos and no difference between DMH and DMH plus IR chrysotile was observed for the primary target organs (intestine and mesothelium).

An audit of the experimental data was conducted for these lifetime carcinogenesis studies of chrysotile asbestos. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these lifetime studies, short-range and intermediate-range chrysotile asbestos did not induce overt toxicity and did not affect survival when ingested at a level of 1% in the diet by male and female F344/N rats. There was *no evidence of carcinogenicity* in male or female rats exposed to SR chrysotile asbestos

or in female rats exposed to IR chrysotile asbestos. There was *some evidence of carcinogenicity* in male rats exposed to IR chrysotile asbestos as indicated by an increased incidence of adenomatous polyps in the large intestine. The cocarcinogenesis studies of 1,2-dimethylhydrazine dihydrochloride and IR chrysotile asbestos were considered inconclusive for determining whether IR chrysotile asbestos had either a tumor-enhancing or protective effect, although an increased incidence of neoplasms was observed in the kidneys of female rats exposed to DMH plus IR chrysotile as compared with those exposed to DMH alone.

Report Date: November 1985

Note: Chrysotile Asbestose was also tested in Syrian Golden Hamsters administered in feed (See TR-246, reported 1990).

TR-296 Toxicology and Carcinogenesis Studies of Tetrakis(hydroxymethyl) phosphonium sulfate (THPS) (CAS No. 55566-30-8) and Tetrakis(hydroxymethyl) phosphonium chloride (THPC) (CAS No. 124-64-1) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of tetrakis (hydroxymethyl)phosphonium sulfate (THPS) and tetrakis (hydroxymethyl)phosphonium chloride (THPC) were conducted because of the widespread use of these chemicals as flame retardants in cotton fabrics. THPS was available as a 72% aqueous solution and THPC as a 75% aqueous solution. Short-term gavage studies with a range of doses were conducted first to identify toxic effects and affected sites and to determine doses for the 2-year studies. The doses selected for the 14-day studies ranged from 12.5 to 200 mg/kg THPS for rats and mice, 9.4 to 150 mg/kg THPC for rats, and 18.8 to 300 mg/kg THPC for mice. Mortality and reduction in body weight gain occurred at the two highest doses in the 14-day studies. There was hind limb paralysis in some rats and mice dosed at the highest concentrations of THPS and THPC.

In the 13-week studies, doses of THPS ranged from 5 to 60 mg/kg in rats and from 2 to 180 mg/kg in mice; doses of THPC ranged from 3.75 to 60 mg/kg in rats and from 1.5 to 135 mg/kg in mice. Mortality and reduction in body weight gain occurred at the two higher doses for both sexes and species. Vascular degeneration of hepatocytes or hepatocellular necrosis was a common histopathologic finding. Hind limb paralysis was noted in rats and mice receiving the highest dose of THPC, and axonal degeneration, characterized by swollen axon sheaths, missing or fragmented axons, and some proliferation of neurolemma cells, was observed in rats. These lesions were found in the sciatic nerve, dorsal roots of the caudal spinal nerves, and the tracts of the spinal cord, particularly in the dorsal column of the lumbar cord.

Two-year studies were conducted in F344/N rats by administering 0, 5, or 10 mg/kg THPS or 0, 3.75, or 7.5 mg/kg THPC in deionized water by gavage to groups of 49 or 50 animals of each sex, 5 days per week for 103 or 104 weeks. Groups of 49 or 50 B6C3F$_1$ mice were administered 0, 5, or 10 mg/kg THPS (each sex), 0, 7.5, or 15 mg/kg THPC (males), or 0, 15, or 30 mg/kg THPC (females).

Survival of male rats was reduced for the low dose (after week 102) and the high dose (after week 67) groups given THPS compared with that of the vehicle controls; survival at terminal kill was as follows: vehicle control, 28/50; low dose, 13/50; high dose, 16/50. Survival of the high dose group of female rats given THPC was lower after week 70 than that of the vehicle controls (survival at terminal kill: 37/50; 34/50; 21/50). Mean body weights of rats dosed with THPS or THPC were comparable to those of the vehicle controls. There was no difference in survival or mean body weights between the vehicle controls and mice dosed with either THPS or THPC. No neurotoxicity or any other signs of clinical toxicity were observed.

A nonneoplastic effect common to 13-week and 2-year exposure to THPS or THPC was an increase in the incidence of hepatocellular lesions, primarily cytoplasmic vacuolization. The incidences of this lesion in the two-year studies were dose related for all studies except for the mice receiving THPS. Other lesions observed included focal hyperplasia of the adrenal medulla in high dose male mice given THPS and follicular cell hyperplasia of the thyroid gland in high dose female mice given THPC. The increased incidences of hematopoietic system lesions observed in these studies were not considered biologically related to chemical exposure because the increases were marginal, no dose-response relationship was observed, and the incidences of these lesions are highly variable in untreated rats and mice.

The incidences of mononuclear cell leukemia in low dose male rats administered THPS or THPC were somewhat greater than those in the vehicle controls (THPS: 30/50; 36/50; 20/50; THPC: 19/50; 25/50; 16/50). Low dose male mice administered THPS had an increased incidence of malignant lymphomas when compared with vehicle controls (2/50; 9/50; 0/50). These marginal increases in the incidences of hematopoietic system tumors were not considered related to chemical exposure, since they were significant only by the life table tests and were not dose related.

THPC demonstrated no mutagenic activity in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation. Both THPS and THPC induced forward mutations in mouse lymphoma L5178Y cells without metabolic activation; neither was tested in the presence of S9. THPC increased the frequency of sister-chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells in the presence and absence of exogenous metabolic activation.

An audit of the experimental data was conducted for the 2-year studies of THPS and THPC. No discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenicity* of THPS in either sex of F344/N rats or B6C3F$_1$ mice given 5 or 10 mg/kg. There was no evidence of carcinogenicity of THPC in either sex of F344/N rats given 3.75 or 7.5 mg/kg, in male B6C3F$_1$ mice given 7.5 or 15 mg/kg, or in female B6C3F$_1$ mice given 15 or 30 mg/kg.

Report Date: February 1987

TR-297 Benzyl Chloride (CAS: 100-44-7)

No Technical Report issued; results of study in journal article.

W Lijinsky, Chronic Bioassay of Benzyl Chloride in F344 Rats and C57BL/6JXBALB Mice. J. Nat'l Cancer Inst 77, No. 4:941-949 (1986).

TR-298 Toxicology and Carcinogenesis Studies of Dimethyl Morpholinophosphoramidate (CAS No. 597-25-1) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Dimethyl morpholinophosphoramidate (DMMPA, greater than 99% pure) was developed for use as a stimulant for the physical (but not biologic) properties of anticholinesterase agents in chemical defense training. Because of the potential for human exposure, the toxicity and carcinogenicity of DMMPA were investigated. Fourteen-day and 13-week studies were conducted to determine short-term toxicity, to identify target organs, and to establish doses for the 2-year toxicology and carcinogenesis studies.

In the 14-day studies, groups of five male and five female F344/N rats were administered DMMPA in corn oil by gavage daily at 0, 313, 1,250, 2,500, or 5,000 mg/kg body weight for 14 consecutive days. All the rats receiving DMMPA at 2,500 or 5,000 mg/kg, except one male receiving 5,000 mg/kg, died before the end of the studies. Rats receiving DMMPA at doses of 1,250 mg/kg or less survived. The final mean body weights of the surviving dosed rats were within ± 8% of those of the vehicle controls. Compound-related gross lesions were not found at necropsy. Groups of five B6C3F$_1$ mice of each sex were given DMMPA by the same route on the same schedule at 0, 250, 500, 1,000, 2,000, or 4,000 mg/kg. All the mice given DMMPA at 2,000 or 4,000 mg/kg died before the end of the studies. Mice administered DMMPA at doses of 1,000 mg/kg or less survived. The final mean body weight of the male mice given DMMPA at 1,000 mg/kg was 14% greater than that of the vehicle controls, whereas that of the other dosed survivors was within 10% of that of the vehicle controls. Compound-related gross lesions were not found at necropsy.

In the 13-week studies, groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice were given DMMPA by gavage in corn oil at 0, 200, 400, 1,200, or 1,600 mg/kg body weight, 5 days per week for 13 weeks. All rats given DMMPA at 400 mg/kg or less survived, and no more than 3/10 died in any of the higher dose groups. The final mean body weights of the dosed male rats were 6% to 11% greater than that of the vehicle controls; weights of the dosed female rats were similar to that of the vehicle controls (-6% to 1%). There was a dose-related increase in liver weight/body weight ratio.

All the mice given DMMPA at 1,600 mg/kg, except one female, died before the end of the 13-week studies; mice receiving DMMPA at 1,200 mg/kg or less survived. The final mean body weights of the dosed male mice were 3.4% to 6.9% greater than that of the vehicle controls. The final mean body weights of dosed female mice and vehicle controls were similar. Compound-related gross or histopathologic changes were not observed in rats or mice.

In the 2-year toxicology and carcinogenesis studies, groups of 50 male and 50 female F344/N rats were given DMMPA in corn oil by gavage at doses of 0, 150, 300, or 600 mg/kg body weight, 5 days per week for 103 weeks. Groups of 50 male B6C3F$_1$ mice were given DMMPA at 0, 150, 300 mg/kg body weight, and groups of 50 female B6C3F$_1$ mice were given DMMPA at 0, 300, 600 mg/kg body weight on the same schedule. Doses of 300 or 600 mg/kg were originally selected for male mice for the 2-year study; because 19/50 high dose male mice died by week 19, all male mice were killed and doses of 0, 150, and 300 mg/kg were selected for the restart of the 2-year study in male mice. The survival of high dose male (22/50) and female (24/50) rats was reduced ($P<0.025$) relative to that of the male (37/50) and female (36/50) vehicle controls. Mean body weights were less than 10% lower in the mid dose and high dose male rats and in the high dose female rats than in the vehicle controls. DMMPA administration did not significantly affect body weight gain or survival of male and female mice.

At the 600 mg/kg dose, increased ($P<0.05$) incidences of mononuclear cell leukemia occurred in both male rats (vehicle control, 14/50; 150 mg/kg, 21/50; 300 mg/kg, 19/50; 600 mg/kg, 25/50) and female rats (9/50; 13/50; 12/49; 18/50). DMMPA-related neoplastic or nonneoplastic lesions were not observed in the dosed mice.

DMMPA was not mutagenic in strains TA100, TA1535, TA1537, or TA98 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9. DMMPA was mutagenic in the L5178Y/TK$^{+/-}$ mouse lymphoma assay in the absence of S9; it was not tested in the presence of S9. DMMPA induced chromosomal aberrations and sister-chromatid exchanges in Chinese hamster ovary cells in the absence of S9, but cytogenetic effects were not observed in the presence of Aroclor 1254-induced rat liver S9.

An audit of the experimental data for these 2-year carcinogenesis studies on DMMPA was conducted. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenicity* for male and female F344/N rats given dimethyl morpholinophosphoramidate, as indicated by increased incidences of mononuclear cell leukemia. There was *no evidence of carcinogenicity* for male and female $B6C3F_1$ mice given dimethyl morpholinophosphoramidate at doses of 150 (male), 300, or 600 (female) mg/kg for 2 years.

Synonyms: dimethyl morpholinophosphonate; phosphonic acid, morpholino, dimethyl ester; DMMPA; phosphonic acid, 4-morpholinyl-, dimethyl ester

Report Date: January 1986

TR-299 Toxicology and Carcinogenesis Studies of C.I. Disperse Blue 1 (A Commercial Dye Containing Approximately 50% 1,4,5,8-Tetraaminoanthraquinone, and 20% Water) (CAS No. 2475-45-8) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

C.I. Disperse Blue 1, a component of several semipermanent hair dyes, was studied as a commercial-grade product (minus lignosulfonate dispersants) containing approximately 50% 1,4,5,8-tetraaminoanthraquinone, 30% other compounds structurally related to 1,4,5,8-tetraaminoanthraquinone, and 20% water. C.I. Disperse Blue 1 was studied for toxicity and carcinogenicity in single-administration gavage, 14-day feed, 13-week feed, and 104-week feed studies. All studies used F344/N rats and $B6C3F_1$ mice.

In the single-administration gavage studies, no deaths occurred within 14 days at doses up to 3,000 mg/kg C.I. Disperse Blue 1 in rats or up to 2,000 mg/kg in mice. In the 14-day studies, rats and mice received dietary concentrations of up to 50,000 ppm. All male rats survived, and 2/5 female rats in the 50,000-ppm group died. All mice receiving 25,000 ppm or more died. Three of five males and 2/5 female mice in the 12,500-ppm groups died.

In the 13-week studies, diets containing concentrations up to 20,000 ppm C.I. Disperse Blue 1 were fed to rats, and diets containing concentrations up to 10,000 ppm were fed to mice. No compound-related deaths of rats occurred; however, pathologic changes occurred at 2,500 ppm and higher and included urinary tract calculi, urinary bladder inflammation, hyperplasia of the urinary bladder transitional epithelium, and nephrosis, Compound-related deaths occurred at 10,000 ppm in mice of each sex. Pathologic changes included chronic inflammation and hyperplasia of the urinary bladder transitional epithelium and urinary tract calculi at dietary concentrations of 2,500 ppm and higher and nephrosis, myocardial necrosis, and testicular degeneration at 10,000 ppm. The renal lesions at 5,000 ppm were considered to be potentially life threatening. These composite findings from the short-term studies were used to identify target organs and to help select dietary concentrations for the longer term studies.

In the 2-year studies in rats, groups of 50 animals of each sex were administered C.I. Disperse Blue 1 at dietary concentrations of 0, 1,250, 2,500, or 5,000 ppm. These dietary concentrations corresponded to 0, 45, 95, and 217 mg/kg per day for males and 0, 56, 111, and 240 mg/kg per day for females. Survival of males and females in the 5,000 ppm groups and males in the 2,500-ppm group was significantly reduced. Final body weights, as percent of controls, were: male—low dose 100%; mid dose, 94%; high dose, 85%; female—low dose, 99%; mid dose, 94%; high dose, 87%.

Compound-related effects of feeding diets containing C.I. Disperse Blue 1 for 104 weeks to F344/N rats included urinary bladder neoplasms and calculi at the incidences noted in the table. Positive statistical associations existed between the presence of calculi and transitional cell neoplasms of the urinary bladder in male and female rats, leiomyomas or leiomyosarcomas (combined) in female rats, and squamous cell neoplasms in male rats.

The increased incidence of pancreatic islet cell adenomas or carcinomas (combined) in high dose male rats was significant by survival-adjusted analyses (overall incidences: control, 1/49; low dose, 2/50; mid dose, 5/50; high dose, 3/50).

In the 2-year studies in mice, 50 animals of each sex were administered diets containing C.I. Disperse Blue 1 at 0, 600, 1,200, or 2,500 ppm. These dietary concentrations corresponded to doses of 0, 112, 239, and 540 mg/kg per day for males and 0, 108, 235, and 520 mg/kg per day for females. Survival was comparable among control and dosed male or female mice. Final body weights, as percent of controls, were as follows: male—low dose, 97%; mid dose, 98%; high dose, 101%; female—low dose, 110%; mid dose, 104%; high dose, 91%.

The incidences of hepatocellular adenomas or carcinomas (combined) were increased for dosed male mice (9/50; 21/50; 16/50) and for low dose female mice (3/50; 13/49; 3/50; 4/50). Alveolar/bronchiolar adenomas or carcinomas (combined) occurred with an increased incidence in high dose male mice (4/50; 9/49; 5/50; 11/50).

Several nonneoplastic effects were detected in the kidneys of mid dose and high dose male and high dose female rats and of all dosed groups of male and female mice. These effects on the kidney included calculi, hydronephrosis, and epithelial hyperplasia in rats and casts and renal tubular degeneration in mice.

C.I. Disperse Blue 1 was studied for mutagenicity in *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9. C.I. Disperse Blue 1 was mutagenic in strain TA1535 in the presence of S9 and in strains TA97 and TA98 in the presence or absence of S9; it was not mutagenic in strain TA100.

An audit of the experimental data was conducted for the 2-year toxicology and carcinogenesis studies of C.I. Disperse Blue 1. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these feed studies of C.I. Disperse Blue 1, there was *clear evidence of car-*

cinogenicity for male and female F344/N rats as shown by the increased occurrence of transitional cell papillomas and carcinomas, of leiomyomas and leiomyosarcomas, and of squamous cell papillomas and carcinomas of the urinary bladder. Urinary bladder calculi were observed in the groups of rats in which urinary bladder neoplasms were increased. Positive associations existed between the presence of calculi and transitional cell neoplasms in male and female rats, leiomyomas or leiomyosarcomas (combined) in female rats, and squamous cell neoplasms in male rats. A marginally increased occurrence of pancreatic islet cell adenomas or carcinomas (combined) was observed in male rats exposed to C.I. Disperse Blue 1. There was *equivocal evidence of carcinogenicity* of C.I. Disperse Blue 1 in male $B6C3F_1$ mice as shown by marginally increased incidences of hepatocellular adenomas or carcinomas (combined) in dosed male mice and a marginally increased occurrence of alveolar/bronchiolar adenomas or carcinomas (combined) in high dose male mice. There was *no evidence of carcinogenicity* of C.I. Disperse Blue 1 in female $B6C3F_1$ mice.

Synonyms: C.I. 64500; 1,4,5,8-tetraamino-9,10-anthracenedione; 1,4,5,8-tetraaminoanthraquinone

Report Date: May 1986

TR-300 Toxicology and Carcinogenesis Studies of 3-Chloro-2-methylpropene (Technical Grade Containing 5% Dimethylvinyl Chloride) (CAS No. 563-47-3) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of technical-grade 3-chloro-2-methylpropene (containing 5% dimethylvinyl chloride), a widely used insecticide and a chemical intermediate, were performed on F344/N rats and $B6C3F_1$ mice. In the 13-week studies, 50%-100% mortality occurred in groups of male and female rats receiving 400 mg/kg, male rats receiving 300 mg/kg, and male and female mice receiving 500-1,250 mg/kg. Inflammation and necrosis of the liver were seen in rats and mice, and necrosis of cortical tubules of the kidney was seen in mice. Based on these observations, groups of 50 male and 50 female rats were administered 3-chloro-2-methylpropene in corn oil by gavage at doses of 0, 75, or 150 mg/kg body weight, 5 days per week for 103 weeks, and groups of 50 male and 50 female mice received 3-chloro-2-methylpropene at 0, 100, or 200 mg/kg on the same schedule.

In the 2-year studies, the mean body weight of high dose male rats was consistently 10%-15% lower than that of the vehicle control group, and late in the study there was a marginal reduction in survival of high dose male rats. Mean body weights and survival in low dose male rats and in both dosed groups of female rats were comparable to those of their vehicle control groups. Mean body weights of high dose male mice and of both dosed groups of female mice were slightly (5%-9%) lower than those of the vehicle controls, whereas survival in both male and female mice was not affected by 3-chloro-2-methylpropene administration.

Dose-related increases in the incidence of forestomach inflammation were observed in male and female mice (male: vehicle control, 0/49; low dose, 9/49; high dose, 7/49; female: vehicle control, 2/50; low dose, 3/48; high dose, 9/44). Increased incidences of forestomach basal cell hyperplasia were observed in rats and mice of each sex. 3-Chloro-2-methylpropene induced forestomach squamous cell papillomas and squamous cell carcinomas in rats and mice as shown in the table. Invasion or metastasis of the squamous cell carcinomas to other organs was observed in two low dose male, three high dose male, and one high dose female mice.

Renal tubular cell adenocarcinomas (1/49), renal transitional cell carcinomas (1/49), and transitional cell papillomas (1/49) of the urinary bladder were observed in high dose male rats, and renal tubular cell adenomas (1/50) and renal tubular cell adenocarcinomas (1/50) were seen in low dose male rats. These urinary tract neoplasms were not observed in vehicle controls.

The incidences of inflammation of the nasal cavity and of nephropathy/nephrosis were greater in the two dosed groups than in the vehicle control groups of rats and mice of each sex.

Negative trends or lower incidences of pheochromocytomas of the adrenal gland and C-cell adenomas or carcinomas (combined) of the thyroid gland were observed in dosed male rats. Negative trends were observed in the incidences of hepatocellular adenomas or carcinomas (combined) in dosed male mice and of hemangiomas or hemangiosarcomas (combined) in dosed female mice.

3-Chloro-2-methylpropene was weakly mutagenic in *Salmonella typhimurium* strain TA1537 with 10% rat liver S9; results in strain TA100 with 10% Syrian hamster liver S9 or with 10% or 30% rat liver S9 were judged equivocal. Mutagenicity tests with *S. typhimurium* strains TA1535 and TA98 were negative with or without metabolic activation. 3-Chloro-2-methylpropene was mutagenic in the mouse lymphoma L5178/TK$^{+/-}$ forward mutation assay without exogenous metabolic activation. Cytogenetics tests with cultured Chinese hamster ovary cells were positive for induction of chromosomal aberrations and sister-chromatid exchanges (SCE's) in the absence of rat liver S9. With metabolic activation, SCE levels remained significantly elevated, but the number of chromosomal aberrations was reduced.

An audit of the experimental data was conducted for these 2-year carcinogenesis studies on 3-chloro-2-methylpropene. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenicity* for 3-chloro-2-methylpropene as shown by the increased incidences of squamous cell neoplasms in the fore-

stomach of male and female F344/N rats and of male and female B6C3F$_1$ mice.

Synonyms: 2-methallyl chloride; methyl allyl chloride; β-methallyl chloride; π-chloroisobutylene; isobutenyl chloride; 3-chloro-2-methyl-1-propene; 2-methyl-2-propenyl chloride

Report Date: June 1986

TR-301 Toxicology and Carcinogenesis Studies of ORTHO-Phenylphenol (CAS No. 90-43-7) Alone and with 7,12-Dimethylbenz(a)anthracene (CAS No. 57-97-6) in Swiss CD-1 Mice (Dermal Studies)

o-Phenylphenol is used primarily as a germicide and fungicide for citrus fruits and vegetables and was selected for carcinogenesis studies because of the potential for human exposure. Four-week studies were conducted in which groups of 10 male and 10 female Swiss Webster mice were given dermal applications to the dorsal interscapular region of 0, 6, 11, 21, 36, or 56 mg of *o*-phenylphenol in 0.1 ml of acetone. Doses were administered 3 days per week for 4 weeks, and animals were monitored for clinical changes. Reductions in body weights of acetone vehicle control were observed, but no compound-related changes in weight or survival occurred in male or female mice administered *o*-phenylphenol. *o*-Phenylphenol caused dose-related ulcerative lesions at the site of application. The severity of these lesions was judged not to be life threatening.

Carcinogenesis studies were conducted to determine whether *o*-phenylphenol was a complete carcinogen for skin or a promoter in a two-stage initiation/promotion skin paint model. Groups of 50 Swiss CD-1 mice of each sex were used for up to 102 weeks. Five dose groups were used: an acetone vehicle control group; a positive control group initiated with 7,12-dimethylbenz(a)anthracene (DMBA) and promoted with 12-O-tetradecanoylphorbol-13-acetate (TPA); an initiator control group that received DMBA plus acetone; a group that received repeated applications of *o*-phenylphenol. The following doses were applied dermally to a clipped area on the dorsal interscapular region 3 days per week: *o*-phenylphenol – 55.5 mg/0.1 ml acetone; or TPA – 0.005 mg/0.1 ml acetone. DMBA was administered as a single dose at a concentration of 0.05 mg/0.1 ml acetone to the dorsal interscapular region.

In the 2-year studies, mean body weights of the *o*-phenylphenol, DMBA/*o*-phenylphenol, and DMBA/TPA groups were not markedly different from those of mice that received DMBA/acetone. Similarly, there were no significant group differences in survival except for a decrease in survival in the positive control group (DMBA/TPA).

Skin neoplasms classified as squamous cell papillomas, squamous cell carcinomas, basal cell tumors, basal cell carcinomas, keratoacanthomas, or sebaceous adenomas occurred in mice dosed with DMBA/acetone, DMBA/*o*-phenylphenol, or DMBA/TPA alone. However, the incidence of skin neoplasms in mice dosed with DMBA/acetone (15/100) was similar to that in mice dosed with DMBA/*o*-phenylphenol (17/100). The incidence of skin neoplasms in male and female mice dosed with DMBA/TPA (52/100) was substantially greater than those in mice dosed with either DMBA/acetone or DMBA/*o*-phenylphenol. Similarly, the mean time of appearance of skin papillomas occurred much earlier in the DMBA/TPA groups than in the DMBA/acetone or DMBA/*o*-phenylphenol groups. All groups had nonneoplastic lesions consisting of inflammation, ulceration, hyperkeratosis, and acanthosis at the site of application. These lesions were present in the acetone vehicle control group and, to a larger extent, in the *o*-phenylphenol, DMBA/*o*-phenylphenol, and DMBA/TPA groups. No skin neoplasms were observed in male or female mice receiving o-phenylphenol or in the acetone vehicle control groups. Moreover, a complete histopathologic review revealed no other neoplasms at any other site at significantly increased incidences in the groups receiving *o*-phenylphenol compared with the acetone vehicle controls. There were also no tumor-enhancing (or tumor-inhibiting) effects of *o*-phenylphenol and DMBA given in combination.

o-Phenylphenol was weakly mutagenic in strains TA1535 of *Salmonella typhimurium* only in the absence of rat liver S9; it was not mutagenic in strains TA1537, TA98, or TA100. It was mutagenic in the mouse lymphoma L5178/TK$^{+/-}$ assay in the presence or absence of Aroclor 1254-induced male F344 rat liver S9. *o*-Phenylphenol did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*. *o*-Phenylphenol induced sister-chromatid exchanges in Chinese hamster ovary (CHO) cells only in the absence of rat liver S9. It did not induce chromosomal aberrations in CHO cells in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9.

An audit of the experimental data was conducted for these 2-year studies of o-phenylphenol. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year dermal application studies, there was *no evidence of carcinogenicity* in male or female Swiss CD-1 mice administered *o*-phenylphenol alone or as a promoter following initiation with DMBA. *o*-Phenylphenol, however, caused nonneoplastic lesions, which included ulceration, inflammation, and hyperkeratosis, at the site of application.

Report Date: March 1986

TR-302 1,2-Epoxyhexadecane (CAS: 7320-37-8)

Study considered inadequate; no Technical Report will be issued.

TR-303 Toxicology and Carcinogenesis Studies of 4-Vinylcyclohexene (CAS No. 100-40-3) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of 4-vinylcyclohexene (greater than 98% pure), a dimer of 1,3-butadiene present in the off-gasses from tire curing, were conducted by administering the chemical in corn oil by gavage 5 days per week at doses of 0, 200, or 400 mg/kg body weight to groups of 50 F344/N rats and B6C3F$_1$ mice of each sex for 103 weeks. Doses selected for the 2-year studies were based on survival, body weight gains, and histopathologic effects observed during the 14-day and 13 week studies.

All rats and most mice in the 14-day studies died when administered doses greater than or equal to 1,250 mg/kg, although no compound-related gross or histopathologic effects were observed. Final body weights were reduced in the 13-week studies in male rats receiving doses greater than or equal to 400 mg/kg of 4-vinylcyclohexene, in female rats receiving 800 mg/kg, and in female mice receiving 600 mg/kg. Extensive mortality was observed only in mice dosed at 1,200 mg/kg. Compound-related histopathologic effects in the 13-week studies included hyaline droplet degeneration of the proximal convoluted tubules of the kidney in dosed male rats, the severity of which was dose related, and a reduction in the number of primary follicles and mature graafian follicles in the ovaries of female mice receiving 1,200 mg/kg of 4-vinylcyclohexene. No compound-related gross or histopathologic effects were evident in dosed female rats or male mice in the 13-week studies.

Many dosed rats died early in the 2-year studies (male: vehicle control, 17/50; low dose, 37/50; high dose, 45/50; female: vehicle control, 10/50; low dose, 22/50; high dose, 36/50; $P<0.001$ for all groups except low dose female rats, for which $P=0.022$). The poor survival of dosed male and female rats reduced the sensitivity of the studies for detecting the possible carcinogenic effects of 4-vinylcyclohexene. Mean body weights of dosed rats were comparable to those of their respective vehicle controls, except for high dose males late in the study. Survival of high dose mice of each sex was lower ($P<0.001$) than that of the vehicle controls, whereas survival of low dose mice of each sex was comparable to that of the vehicle controls. Mean body weights of high dose mice of each sex were generally lower than those of the vehicle controls throughout most of the 2-year studies.

Administration of 4-vinylcyclohexene to F344/N rats by gavage for 2 years was associated with a slightly increased incidence of epithelial hyperplasia of the forestomach (1/50; 3/50; 5/47) and squamous cell papillomas or carcinomas (combined) of the skin in high dose males (0/50; 1/50; 4/50). Low dose female rats, whose survival was more similar to that of the vehicle controls, had a marginally increased incidence of adenomas or squamous cell carcinomas (combined) of the clitoral gland (1/50; 5/50; 0/49).

In B6C3F$_1$ mice, administration of 4-vinylcyclohexene for two years by gavage was associated with mild, acute inflammatory lesions and epithelial hyperplasia of the forestomach, especially in males (0/47; 7/50; 7/46), and with an increased incidence of a number of other nonneoplastic lesions, including lung congestion in high dose males and females, splenic red pulp atrophy in high dose males, congestion of the adrenal gland in high dose females, and cytologic alteration of the adrenal cortex in low dose and high dose females.

The incidences of uncommon ovarian neoplasms were markedly increased ($P<0.01$) in both groups of dosed female mice (mixed tumor, benign: 0/49; 25/48, 52%; 11/47, 23%; granulosa cell tumor or carcinoma [combined]: 1/49, 2%; 10/48, 21%; 13/47, 28%). In addition, a slight increase in the incidence of adrenal gland adenomas in high dose females was observed (0/50; 3/49, 6%; 4/48, 8%). The extensive mortality seen in the high dose male mice confounded interpretation of the increased incidences of malignant lymphomas and alveolar/bronchiolar adenomas or carcinomas (combined) of the lung seen in these animals surviving to the end of the study (malignant lymphomas: 3/37, 8%; 5/39, 13%; 4/7, 57%; alveolar/bronchiolar adenomas or carcinomas [combined]: 3/37, 8%; 9/39, 23%; 3/7, 43%).

4-Vinylcyclohexene was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9 when tested according to the preincubational protocol. However, several of its metabolites, including 4-vinylcyclohexene diepoxide, have been shown to be mutagenic in Salmonella and/or induce chromosomal damage in vitro.

An audit of the experimental data was conducted for these 2-year carcinogenesis studies on 4-vinylcyclohexene. No data discrepancies were found that influenced the final interpretations.

4-Vinylcyclohexene was administered by gavage in corn oil to F344/N rats and B6C3F$_1$ mice of each sex at doses of 200 or 400 mg/kg for 103 weeks. Under these conditions, the 2-year gavage studies of 4-vinylcyclohexene in male and female rats and male mice were considered *inadequate studies of carcinogenicity* because of extensive and early mortality at the high dose or at both doses and the lack of conclusive evidence of a carcinogenic effect. There was *clear evidence of carcinogenicity of* 4-vinylcyclohexene for female mice, as shown by markedly increased incidences of uncommon ovarian neoplasms at both doses. In addition, the increased incidence of adrenal gland adenomas in high dose female mice may have been related to the administration of 4-vinylcyclohexene.

Synonym: 4-ethenylcyclohexene

Report Date: August 1986

TR-304 Toxicology and Carcinogenesis Studies of Chlorendic Acid (CAS No. 115-28-6) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Chlorendic acid is a chemical intermediate used in the preparation of fire-retardant polyester resins and plasticizers. Toxicology and carcinogenesis studies of chlorendic acid (greater than 98% pure) were conducted by administering the chemical in feed tc groups of 50 male and 50 female F344/N rats and B6C3F$_1$ mice at concentrations of 0, 620, or 1,250 ppm for 103 weeks. The estimated mean daily consumption of chlorendic acid was 27 and 56 mg/kg body weight for low dose and high dose male rats and 39 and 66 mg/kg for low dose and high dose female rats. In mice, the estimated daily consumption was 89 and 185 mg/kg for low dose and high dose males and 100 and 207 mg/kg for low dose and high dose females. These concentrations were selected because higher levels in the 14-day and 13-week studies caused decreased mean body weights, more deaths, and increased incidences of liver lesions (rats: centrilobular cytomegaly, mitotic alterations, bile duct hyperplasia; mice: centrilobular cytomegaly, mitotic alterations, coagulative necrosis) relative to control groups.

Survival and feed consumption of dosed male and female rats and mice in the 2-year studies were similar to those of controls. Mean body weights of high dose male and female rats and mice were lower than those of controls. Mean body weights of high dose female rats were 16%-24% lower than those of controls during the second half of the study.

In the 2-year chlorendic acid feed studies, incidences of nonneoplastic lesions of the liver in dosed male rats (cystic degeneration) and dosed female rats (granulomatous inflammation, pigmentation, and bile duct hyperplasia) were increased. The incidences of neoplastic nodules of the liver were significantly increased in dosed male rats (control, 2/50; low dose, 21/50; high dose, 23/50) and high dose female rats (1/50; 3/39; 11/50). The incidence of hepatocellular carcinomas was also increased in high dose female rats (0/50; 3/49; 5/50). In mice, the incidences of nonneoplastic lesions of the liver were increased in dosed males (coagulative necrosis) and high dose females (mitotic alterations). The incidences of hepatocellular adenomas (5/50; 9/49; 10/50), hepatocellular carcinomas (9/50; 17/50; 20/50), and hepatocellular adenomas or carcinomas (combined) (13/50; 23/49; 27/50) were increased in dosed male mice. Hepatocellular carcinomas metastasized to the lung in 2/50 control, 4/49 low dose, and 7/50 high dose male mice. Hepatocellular adenomas or carcinomas (combined) were not significantly increased in female mice (3/50; 7/49; 7/50).

The incidences of acinar cell hyperplasia (0/49; 4/50; 4/50) and acinar cell adenomas (0/49; 4/50; 6/50) of the pancreas were increased in dosed male rats relative to those of controls. Pancreatic acinar cell adenoma is an uncommon neoplasm in untreated control F344/N rats in NTP studies (3/1,667).

In dosed male rats, incidences of alveolar/bronchiolar adenomas of the lung (0/50; 3/50; 5/50) were increased. The incidences of alveolar/bronchiolar adenomas or carcinomas (combined) in dosed female mice were also increased (1/50; 5/50; 6/50). Preputial gland carcinomas occurred at a greater incidence in low dose male rats (1/50; 8/50; 4/50) than in controls. An adenoma and a squamous cell papilloma were observed in two low dose male rats. The incidences of sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) of the salivary gland (1/50; 2/49; 4/50) were increased in dosed male rats. The incidences in the dosed groups were not significantly different from that in the controls, but these tumors are uncommon in F344/N rats receiving no treatment (3/1,689).

Chlorendic acid was not mutagenic in strains TA100, TA98, TA1535, or TA 1537 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver activation when tested according to the preincubational protocol. Chlorendic acid was mutagenic in the L5178Y/TK$^{+/-}$ mouse lymphoma cell forward assay (in the absence of activation) at a dose resulting in toxicity.

An audit of the experimental data was conducted for the 2-year studies of chlorendic acid. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenicity* of chlorendic acid for male F344/N rats as shown by increased incidences of neoplastic nodules of the liver and acinar cell adenomas of the pancreas. Increased incidences of alveolar/bronchiolar adenomas and preputial gland carcinomas may also have been related to the administration of chlorendic acid. There was *clear evidence of carcinogenicity* of chlorendic acid for female F344/N rats as shown by increased incidences of neoplastic nodules and of carcinomas of the liver. There was *clear evidence of carcinogenicity* of chlorendic acid for male B6C3F$_1$ mice as shown by increased incidences of hepatocellular adenomas and of hepatocellular carcinomas. There was *no evidence of carcinogenicity* of chlorendic acid for female B6C3F$_1$ mice given chlorendic acid in the diet at concentrations of 620 or 1,250 ppm for 103 weeks.

Synonym: 1,4,5,6,7,7-hexachloro-5-norbornene-2,3-dicarboxylic acid

Report Date: April 1987

TR-305 Toxicology and Carcinogenesis Studies of Chlorinated Paraffins (C_{23}, 43% Chlorine) (CAS No. 108171-27-3) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of chlorinated paraffins (C_{23}, 43% chlorine), an extreme-pressure lubricant and flame retardant, were conducted by administering the chemical in corn oil by gavage to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex, 5 days per

week for 103 weeks. Additional groups of 10 rats per sex and dose were examined at 6 and at 12 months. Male rats received doses of 0, 1,875, or 3,750 mg/kg body weight; female rats were given 0, 100, 300, or 900 mg/kg. Male and female mice received 0, 2,500, or 5,000 mg/kg. Doses selected for the 2-year studies were based on the results from 13-week studies in which rats of each sex received 0 to 3,750 mg/kg, and mice of each sex, 0 to 7,500 mg/kg. No toxicity of chlorinated paraffins (C_{23}, 43% chlorine) was observed in male rats or in male or female mice in the 13-week studies. A dose-related inflammation of the liver was observed in female rats in the 13-week studies and in male and female rats in the 13-week studies and in male and female rats at 6 and 12 months in the 2-year studies.

Chlorinated paraffins (C_{23}, 43% chlorine) administration did not influence mean body weights of rats during the 2-year studies, but both male and female low dose mice gained less weight than did vehicle controls or the high dose groups. Survival of dosed and vehicle control groups was similar for each sex and species (male rats: vehicle control, 30/50; low dose, 32/50; high dose, 27/50; female rats: 34/50; 30/50; 33/50; 31/50; male mice: 29/50; 36/50; 28/50; female mice: 21/50; 22/50; 20/50). For female mice, 60%-70% of the early deaths in each group were attributed to utero-ovarian infection. The lower survival for female mice may have decreased the sensitivity of this study to detect a carcinogenic effect.

Pheochromocytomas of the adrenal gland medulla occurred with an increased incidence in female rats exposed to chlorinated paraffins (C_{23}, 43% chlorine) (vehicle control, 1/50; low dose, 4/50; mid dose, 6/50; high dose, 7/50). However, adrenal gland medullary hyperplasia was not increased (6/50; 3/50; 1/50; 6/50). Malignant lymphomas were increased in dosed male mice (6/50; 12/50; 16/50). High dose female mice showed a marginal increase in the incidence of hepatocellular carcinomas (1/50; 1/49; 6/50) and in the incidence of adenomas or carcinomas (combined) (4/50; 3/49; 10/50).

The primary nonneoplastic lesion associated with chlorinated paraffins (C_{23}, 43% chlorine) administration was a diffuse lymphohistiocytic inflammation in the liver and in the pancreatic and mesenteric lymph nodes of male and female rats. Splenic congestion was a secondary effect. These lesions occurred earlier and at lower doses in female rats than in male rats. No significant nonneoplastic lesions were considered compound related in mice.

Chlorinated paraffins (C^{23}, 43% chlorine) was not mutagenic in strains TA100, TA1535, TA97, or TA98 of Salmonella typhimurium in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9 when assayed according to the preincubation protocol.

An audit of the experimental data was conducted for these 2-year studies of chlorinated paraffins (C_{23}, 43% chlorine). No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenicity* of chlorinated paraffins (C_{23}, 43% chlorine) for male F344/N rats given 1,875 or 3,750 mg/kg per day. There was *equivocal evidence of carcinogenicity* of chlorinated paraffins (C_{23}, 43% chlorine) for female F344/N rats as shown by an increased incidence of adrenal gland medullary pheochromocytomas. There was *clear evidence of carcinogenicity* of chlorinated paraffins (C_{23}, 43% chlorine) for male B6C3F$_1$ mice as shown by an increase in the incidence of malignant lymphomas. There was *equivocal evidence of carcinogenicity* of chlorinated paraffins (C_{23}, 43% chlorine) for female B6C3F$_1$ mice as shown by a marginal increase in the incidence of hepatocellular neoplasms.

Report Date: May 1986

*The Chemical Abstract Service Service (CAS) number that appeared on this technical report at the time of publication (63449-39-8) reflects the generic CAS number for chlorinated paraffins. This number has been replaced in the NTP Chemtrack chemical tracking system with the more appropriate number.

TR-306 Toxicology and Carcinogenesis Studies of Dichloromethane (Methylene Chloride) (CAS No. 75-09-2) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

Dichloromethane is widely used in industrial processes, food preparation, and agriculture. In industry, dichloromethane is used as a solvent in paint removers, degreasing agents, aerosol propellants, and triacetate solutions; as a blowing agent in flexible urethane foams; and as a process solvent in the manufacture of steroids, antibiotics, vitamins, and tablet coatings. The use of dichloromethane as an extraction solvent for spice oleoresins, hops, and caffeine from coffee has been approved by the U.S. Food and Drug Administration. Dichloromethane has been used as an inhalation anesthetic and as a fumigant for grain and strawberries.

Toxicology and carcinogenesis studies of dichloromethane (99% pure) were conducted by inhalation exposure of groups of 50 male and 50 female F344/N rats and B6C3F$_1$ mice 6 hours per day, 5 days per week, for 102 weeks. The exposure concentrations used (0, 1,000, 2,000, or 4,000 ppm for rats and 0, 2,000, or 4,000 ppm for mice) were selected on the basis of results from 13-week inhalation studies in which groups of 10 rats and 10 mice of each sex were exposed to dichloromethane at concentrations of 525-8,400 ppm 6 hours per day, 5 days per week.

During the 2-year studies in rats, body weight gains for exposed males and females were comparable to those of the chamber controls. The survival of exposed male rats was comparable to that of the chamber controls; however, the survival of all groups of males at the termination of the study was low (control, 16/50; low dose, 16/50; mid dose, 17/50; high dose, 9/50). Most of the early deaths among male rats occurred during the final weeks of the study; the survival of male rats through week 86 of

the study was 36/50, 39/50, 37/50, and 33/50. This decreased survival is believed to be related to the high incidence of leukemia (34/50; 26/50; 32/50; 35/50). Survival of female rats exposed at 4,000 ppm was reduced relative to that of the chamber controls (30/50; 22/50; 22/50; 15/50); leukemia occurred frequently in all female rat groups. Final mean body weights of high dose male mice and low and high dose female mice were 10%-17% lower than those of the chamber controls; these reductions occurred during the last 16 weeks of the study. The survival of dosed male mice and high dose female mice was reduced relative to that of the chamber controls (male: control, 39/50; low dose, 24/50; high dose, 11/50; female: 25/50; 25/50; 8/50). This reduced survival may have been due to the chemically induced development of liver and lung neoplasia in male and female mice.

Increased incidences of benign mammary gland lesions (adenomas and fibroadenomas) occurred in male and female rats exposed to dichloromethane (male: 0/50; 0/50; 2/50; 5/50; female: 5/50; 11/50; 13/50; 23/50). The incidence of malignant mammary gland neoplasms was not increased in female rats (2/50; 2/50; 2/50; 0/50); none was observed in male rats. In addition, integumentary system tumors in the area of the mammary chain occurred with a positive trend in male rats (subcutaneous tissue fibroma or sarcoma: 1/50; 1/50; 2/50; 5/50); the combined incidence of all tumors in the mammary area in male rats was 1/50, 1/50, 4/50, and 9/50.

Exposure to dichloromethane was associated with increased incidences of hepatic hemosiderosis, cytomegaly, cytoplasmic vacuolization, necrosis, granulomatous inflammation, and bile duct fibrosis in both male and female rats. There was a positive but marginal trend in the incidence of hepatocellular neoplastic nodules or hepatocellular carcinomas (combined) in female rats (2/50; 1/50; 4/50; 5/50). The incidence of squamous metaplasia of the nasal cavity was increased in female rats exposed at 4,000 ppm (1/50; 2/50; 3/50; 9/50) but not in males (4/50; 5/50; 3/50; 3/50). No nasal cavity tumors were observed in rats. The increased incidences of mononuclear cell leukemia in mid dose and high dose female rats (17/50; 17/50; 23/50; 23/50) were statistically significant by age-adjusted analyses. In male rats, mesotheliomas (arising primarily from the tunica vaginalis) occurred at increased incidences (0/50; 2/50; 5/50; 4/50).

Lung tumors occurred at increased incidences in male and female mice exposed to dichloromethane (alveolar/bronchiolar adenomas: male – 3/50; 19/50; 24/50; female – 2/50; 23/48; 28/48; alveolar/bronchiolar carcinomas: male – 2/50; 10/50; 28/50; female – 1/50; 13/48; 29/48). Cytologic degeneration of the liver was observed at increased incidences in high dose male and dosed female mice (male: 0/50; 0/49; 22/49; female: 0/50; 23/48; 21/48). Incidences of hepatocellular adenomas or hepatocellular carcinomas (combined) were increased in high dose male and dosed female mice (male: 22/50; 24/49; 33/49; female: 3/50; 16/48; 40/48). There were also dose-related increases in the numbers of mice bearing multiple lung or liver neoplasms. Dose-related increases were observed in the incidences of testicular atrophy in male mice and uterine and ovarian atrophy in female mice; these effects are considered to be secondary responses to neoplasia.

An audit of the experimental data was conducted for the 2-year studies of dichloromethane. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these inhalation studies, there was *some evidence of carcinogenicity* of dichloromethane for male F344/N rats as shown by an increased incidence of benign neoplasms of the mammary gland. There was *clear evidence of carcinogenicity* of dichloromethane for female F344/N rats as shown by increased incidences of benign neoplasms of the mammary gland. There was *clear evidence of carcinogenicity* of dichloromethane for male and female $B6C3F_1$ mice, as shown by increased incidences of alveolar/bronchiolar neoplasms and of hepatocellular neoplasms.

Synonyms: DCM; methylene chloride

Report Date: January 1986

TR-307 Toxicology and Carcinogenesis Studies of Ephedrine Sulfate (CAS No. 134-72-5) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Ephedrine sulfate is a sympathomimetic amine that affects both the central and peripheral nervous systems. An effective bronchiodilator and weak vasoconstrictor, ephedrine sulfate is used extensively in nonprescription pharmaceutical preparations such as nose drops, cold tablets, cough syrups, and, in particular, asthma relief medicines. Ephedrine sulfate was nominated for carcinogenesis studies by the National Cancer Institute because of its widespread and long-term use for the relief of symptoms associated with asthma.

In 14-day repeated-exposure studies, F344/N rats of each sex received diets containing 0-1,500 ppm ephedrine sulfate or drinking water containing 0-1,200 ephedrine sulfate; $B6C3F_1$ mice received diets or drinking water containing 0-5,000 ppm ephedrine sulfate. In the feed studies, the average feed consumption by dosed rats and mice was comparable to that of their respective controls. The average water consumption by rats and mice decreased with increasing concentration of ephedrine sulfate in the drinking water. Thus, subsequent studies used the feed route of administration.

Doses for the 2-year studies were selected on the basis of results from 13-week studies in which F344/N rats of each sex were given diets containing 0, 125, 250, 500, 1,000, or 2,000 ppm ephedrine sulfate and $B6C3F_1$ mice of each sex were given diets containing 0, 310, 630, 1,250, 2,500, or 5,000 ppm ephedrine sulfate. The major response that occurred during the 13-week studies was compound-associated reduction in weight gain. Toxicology and carcinogenesis studies of ephedrine sulfate were conducted by administering diets containing 0, 125, or

250 ppm ephedrine sulfate to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex for 103 weeks. The estimated average amount of ephedrine sulfate consumed per day during the 2-year study was 4 mg/kg and 9 mg/kg for low dose and high dose male rats, 5 mg/kg and 11 mg/kg for female rats, 14 mg/kg and 29 mg/kg for male mice, and 12 mg/kg and 25 mg/kg for female mice.

Survival of chemically exposed female rats during the 2-year study was greater than that of the controls (control, 27/50; low dose, 39/50; high dose, 39/50); survival of exposed male rats and male and female mice was comparable to that of controls. Throughout most of the 2-year studies, mean body weights of rats and mice of each sex receiving diets containing ephedrine sulfate were lower than those of controls.

Neoplasms that occurred in these studies were not considered to be related to administration of ephedrine sulfate. Two high dose female mice had ovarian granulosa cell tumors, and luteomas were found in one low dose and one high dose female mouse. Because of the low incidence, these uncommon, benign tumors could not be clearly related to ephedrine sulfate administration.

Ephedrine sulfate was not mutagenic in four strains of *Salmonella typhimurium* (TA100, TA1535, TA97, or TA98) with or without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 activation. Ephedrine sulfate did not induce sister-chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells.

An audit of the experimental data was conducted for these 2-year studies of ephedrine sulfate. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these studies, there was *no evidence of carcinogenicity* for F344/N rats or B6C3F$_1$ mice of either sex receiving 125 or 250 ppm ephedrine sulfate in the diet for 2 years.

Report Date: May 1986

TR-308 Toxicology and Carcinogenesis Studies of Chlorinated Paraffins (C_{12}, 60% Chlorine) (CAS No. 108171-26-2*) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis assessments of chlorinated paraffins (C_{12}, 60% chlorine), a material widely used as a flame retardant and extreme-pressure lubricant, were conducted in male and female F344/N rats and male and female B6C3F$_1$ mice in single-administration, 16-day, 13-week, and 2-year studies. Doses used in the 2-year studies were 0, 312, or 625 mg/kg body weight per day administered by gavage in corn oil five times per week to groups of 70 male and female rats and 0, 125, or 250 mg/kg administered to groups of 50 male and female mice. Ten male and 10 female rats were killed after 6 and 12 months of dosing and examined for toxicity.

No chemically related toxicity was observed in single-administration studies in which male and female rats received doses of chlorinated paraffins (C_{12}, 60% chlorine) up to 13,600 mg/kg body weight and male and female up to 27,200 mg/kg. In 16-day studies, deaths did occur in groups of male and female rats given 7,500 mg/kg and in groups of male and female mice given doses of 1,875 mg/kg or higher. In 13-week studies, no chemically related deaths occurred among male and female rats given up to 5,000 mg/kg or mice given up to 2,000 mg/kg. Increased liver weights were noted in dosed rats and mice of each sex in the short-term studies, and dosed male rats showed more severe nephropathy than did vehicle controls. Doses selected for the 2-year studies were those that caused a minimal increase in liver weight in the short-term studies.

Liver and kidney weights were increased in dosed rats killed at 6 and 12 months. Morphometric measurements demonstrated hepatocyte hypertrophy in the livers of dosed rats. Lesions of the kidney tubules and interstitial inflammation increased with dose in male and female rats.

During the 2-year studies, body weights of high dose male rats were 8%-12% lower than those of vehicle controls after week 20, and body weights of dosed female mice were about 10% lower than those of vehicle controls during the second year. Survival of dosed male rats was lower than that of vehicle controls after about week 85, perhaps due to toxicity to the kidney (final survival: vehicle control, 27/50; low dose, 6/50; high dose, 3/50). Survival of low dose female rats was lower than that of vehicle controls (34/50; 24/50; 29/50). Survival of dosed male mice was not significantly different from that of vehicle controls (34/50; 31/50; 31/50). Survival of high dose female mice was lower than that of vehicle controls after about week 75 (final survival: 36/50; 31/50; 25/50).

Chemically related nonneoplastic lesions consisted of hypertrophy and minimal focal necrosis of the liver in rats; erosion, inflammation, and ulceration of the glandular stomach and forestomach a in male rats; and formation of multiple cysts in the kidney tubules of male rats. The incidence of nephropathy was also increased in dosed female rats and mice. The maximum tolerated dose may have been exceeded in male and female rats.

Neoplastic lesions associated with chlorinated paraffins (C_{12}, 60% chlorine) administration were found in the liver of rats and mice of each sex (see table p. 12 of Technical Report)

Dosed male rats showed increased incidences of kidney tubular cell hyperplasia (1/50; 9/50; 12/49) and of tubular cell adenomas (0/50; 7/50; 3/49); two low dose males had tubular cell adenocarcinomas. The incidences of mononuclear cell leukemia were increased in dosed male rats (7/50; 12/50; 14/50) and in low dose female rats (11/50; 22/50; 16/50). Pancreatic acinar cell tumors occurred at increased incidences in low dose male rats (11/50; 22/50; 17/50). Follicular cell adenomas or carcinomas (combined) of the thyroid gland were found at increased incidences in both female rats (0/50; 6/50; 6/50) and female mice (8/50; 12/49; 15/49).

Chlorinated paraffins (C_{12}, 60% chlorine) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535 in the presence or absence of Aroclor 1254-induced male Sprague-Dawley or male Syrian hamster liver S9 when tested according to the preincubational protocol.

An audit of the experimental data was conducted for these 2-year studies on chlorinated paraffins (C_{12}, 60% chlorine). No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenicity* of chlorinated paraffins (C_{12}, 60% chlorine) for F344/N rats based on increased incidences of hepatocellular neoplasms (primarily neoplastic nodules) in male and female rats, of adenomas or adenocarcinomas (combined) of the kidney tubular cells in male rats, and of follicular cell adenomas or carcinomas (combined) of the thyroid gland in female rats. Mononuclear cell leukemia in dosed male rats may have been related to administration of chlorinated paraffins (C_{12}, 60% chlorine). There was *clear evidence of carcinogenicity* of chlorinated paraffins (C_{12}, 60% chlorine) for B6C3F$_1$ mice as shown by increased incidences of hepatocellular adenomas and of adenomas or carcinomas (combined) in dosed male and female mice and increased incidences of adenomas and of adenomas or carcinomas (combined) of thyroid gland follicular cells in dosed female mice.

Report Date: May 1986

*The Chemical Abstract Service Service (CAS) number that appeared on this technical report at the time of publication (63449-39-8) reflects the generic CAS number for chlorinated paraffins. This number has been replaced in the NTP Chemtrack chemical tracking system with the more appropriate number.

TR-309 Toxicology and Carcinogenesis Studies of Decabromodiphenyl Oxide (CAS No. 1163-19-5) In F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Toxicology and carcinogenesis studies of decabromodiphenyl oxide, a flame retardant for plastics and other materials, were conducted by exposing groups of 50 male and 50 female F344/N rats and B6C3F$_1$ mice at 0, 25,000, and 50,000 ppm in the diet for 103 weeks. These concentrations were selected because no toxicity was observed at any dose in the 14-day or 13-week studies and 50,000 ppm chemical in the diet is considered to be the highest dose to which rats and mice can be exposed for extended periods of time without reducing the nutritional value of the diet. No compound-related gross or microscopic pathologic effects were observed in the 14-day or 13-week studies.

Body weights of dosed male and female rats and mice in the 2-year studies were comparable to those of the controls. Decreased survival of low dose male rats was not believed to be compound related. No other effects on survival were observed in the 2-year studies. Loss of control male mice (presumably due to fighting) was significant during the first part of the study.

In the 2-year studies, nonneoplastic lesions were observed at increased incidences in rats and mice of each sex. Thrombosis and degeneration of the liver, fibrosis of the spleen, and lymphoid hyperplasia were observed in high dose male rats. Degeneration of the eye was observed in low dose female rats. Nonneoplastic lesions observed in dosed mice were granulomas in the liver of low dose males and hypertrophy in the liver of low dose and high dose males. Follicular cell hyperplasia was observed in thyroid glands of dosed male mice (control, 2/50; low dose, 10/50; high dose, 19/50).

The incidences of neoplastic nodules in the liver of low and high dose male rats (1/50; 7/50; 15/49) and high dose female rats (1/50; 3/49; 9/50) were significantly greater than those in the controls. Mononuclear cell leukemia occurred in dosed male rats with a positive trend (30/50; 33/50; 35/50); this marginal increase was not considered biologically significant. Acinar cell adenomas were observed in the pancreas of four high dose male rats, and a sarcoma was observed in the spleen of one low dose and one high dose male rat. Hepatocellular adenomas or carcinomas (combined) occurred at marginally increased incidences in dosed male mice (8/50; 22/50; 18/50). The incidences of thyroid gland follicular cell adenomas or carcinomas (combined) were increased in dose male mice (0/50; 4/50; 3/50).

A study of decabromodiphenyl oxide absorption from the gastrointestinal tract indicated that absorption was minimal, possibly less than 1%, at the doses administered in the 2-year studies. Additional chemical analysis indicated than the decabromodiphenyl oxide used in these studies contained several less brominated diphenyl oxides. Therefore, since absorption and toxicity of minor impurities are unknown, effects observed in these studies must be attributed to the approximately 95% pure preparation used rather than to pure decabromodiphenyl oxide.

Decabromodiphenyl oxide was not mutagenic in strains TA1535, TA1537, TA98, or TA100 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced Sprague-Dawley male rat or Syrian hamster liver S9 when tested according to the preincubational protocol. Decabromodiphenyl oxide was not mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay in the presence or absence of Aroclor 1254-induced F344/N male rat liver S9. Decabromodiphenyl oxide did not induce sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in vitro in the presence or absence of S9 prepared from livers of Aroclor 1254-induced male Sprague-Dawley rats.

An audit of experimental data was conducted for these 2-year studies on decabromodiphenyl oxide. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year feed studies of decabromodiphenyl oxide, there was *some evidence of*

carcinogenicity for male and female F344/N rats as shown by increased incidences of neoplastic nodules of the liver in low dose (25,000 ppm) males and high dose (50,000 ppm) groups of each sex. There was *equivocal evidence of carcinogenicity* for male $B6C3F_1$ mice as shown by increased incidences of hepatocellular adenomas or carcinomas (combined) in both dosed groups. There was *no evidence of carcinogenicity* for female $B6C3F_1$ mice receiving 25,000 or 50,000 ppm in the diet. Several nonneoplastic lesions were observed at increased incidences, the most notable being thyroid gland follicular cell hyperplasia in male mice.

Synonyms: decabromodiphenyl ether; bis(pentabromophenyl)ether; DBDPO

Report Date: May 1986

TR-310 Toxicology and Carcinogenesis Studies of Marine Diesel Fuel (NO CAS) and JP-5 Navy Fuel (CAS No. 8008-20-6) in $B6C3F_1$ Mice (Dermal Studies)

Toxicology and carcinogenesis studies were conducted by applying marine diesel fuel or JP-5 navy fuel to clipped dorsal interscapular skin of male and female $B6C3F_1$ mice to determine both systemic and dermal effects. Doses for the 2-year studies were set by conducting 14-day and 13-week studies. Doses of 2,000-40,000 mg/kg marine diesel fuel were applied neat in the 14-day studies; in the 13-week studies, doses of 250-4,000 mg/kg marine diesel fuel in acetone were applied with a dose volume of 0.1 ml. Doses of 5,000-40,000 mg/kg JP-5 navy fuel in ethanol were applied in the 14-day studies with a dose volume of 0.5 ml; in the 13-week studies, doses of 500-8,000 mg/kg JP-5 navy fuel in acetone were applied with a dose volume of 0.2 ml. For the 2-year studies, doses were selected which did not cause deaths, decrease body weight gain, or produce excessive dermatitis in the 14-day or 13-week studies. Two-year studies were conducted by administering marine diesel fuel or JP-5 navy fuel by dermal application to groups of 49 or 50 male and 50 female $B6C3F_1$ mice at doses of 0, 250, or 500 mg/kg in an acetone vehicle with a dose volume of 0.1 ml.

Both sexes of mice dosed with 500 mg/kg marine diesel fuel (84-week exposure) and female mice dosed with 500 mg/kg JP-5 navy fuel (90-week exposure) were killed early because of excessive irritation and ulceration at the site of application and to prevent the spread of infection. Survival rates at those times were 26/50 males and 29/50 females dosed with marine diesel fuel and 17/50 females dosed with JP-5 navy fuel. Survival rates at the end of the studies (104 weeks) were reduced ($P<0.01$) in low dose female mice receiving marine diesel fuel (40/50 in vehicle controls compared with 12/50 in the low dose group) or with JP-5 navy fuel (44/50 in vehicle controls compared with 33/50 in the low dose group). Body weight gain was decreased below that of the vehicle controls after week 30 in all groups of mice receiving marine diesel fuel and in both sexes of mice receiving the high dose of JP-5 navy fuel.

There was a marked increase in the incidence of chronic dermatitis in mice receiving marine diesel fuel or JP-5 navy fuel. Chronic dermatitis was defined as a composite lesion of epidermal histopathologic changes generally consisting of acanthosis, hyperkeratosis, and in some instances necrosis and ulceration of the overlying epidermis. Dermal changes frequently included fibrosis, increased amounts of melanin, and the presence of acute and chronic inflammatory cell infiltrates. A dose-related, proportional increase in the severity of the lesions was twofold to threefold greater in the dosed groups than in the vehicle controls. The average degree of severity of the lesions was judged to be minimal in the vehicle controls, mild in the low dose groups, and moderate in the high dose groups of mice dosed with marine diesel fuel or JP-5 navy fuel. There were similar responses at the site of inguinal skin to which the chemicals had migrated after application, but the degree of severity of the lesions was judged to be minimal to mild in the vehicle control and dosed groups of mice.

Squamous cell papillomas or carcinomas (combined) occurred with a positive trend ($P<0.05$) at the site of application in male mice administered marine diesel fuel (vehicle control, 0/49; low dose, 0/49; high dose, 3/49). The total numbers of mice with squamous cell papillomas or carcinomas (combined) both for the site of application and the adjacent inguinal skin were 1/50, 2/49, and 3/50 for the vehicle control, low dose, and high dose groups of male mice and 0/50, 1/45, and 2/48 for female mice. There are no NTP historical data for $B6C3F_1$ mice that received acetone by dermal application. The NTP historical incidence of squamous cell papillomas or carcinomas (combined) in untreated male and female $B6C3F_1$ mice is 0.3%-0.4% in over 3,500 observations.

Marine diesel fuel was not mutagenic in *Salmonella typhimurium* TA98, TA100, TA1535, or TA1537, and JP-5 navy fuel was not mutagenic in strains TA97, TA98, TA100, or TA1535 in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster S9 when tested according to the preincubation protocol.

Audits of the experimental data were conducted for these 2-year studies on marine diesel fuel and JP-5 navy fuel. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year dermal studies, marine diesel fuel at doses of 250 and 500 mg/kg resulted in dose-related increased incidences of squamous cell neoplasms of the skin (primarily carcinomas), providing *equivocal evidence of carcinogenicity* for male and female $B6C3F_1$ mice. The sensitivity for detecting systemic carcinogenicity in female mice dosed with marine diesel fuel was reduced by poor survival. Under the conditions of these 2-year dermal studies, JP-5 navy fuel at doses of 250 and 500 mg/kg provided *no evidence of carcinogenicity* for male and female $B6C3F_1$ mice.

Report Date: September 1986

TR-311 Toxicology and Carcinogenesis Studies of Tetrachloroethylene (Perchloroethylene) (CAS No. 127-18-4) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

Tetrachloroethylene is used primarily as a dry cleaning agent, an industrial solvent for fats, oils, tars, rubber, and gums, and a metal degreasing agent. Tetrachloroethylene had antihelminthic uses, particularly for hookworms (1.6-8 g/60 kg), and was formerly used in combination with some grain protectants and fumigants.

Toxicology and carcinogenesis studies of tetrachloroethylene (99.9% pure) were conducted by inhalation exposure of groups of 50 male and 50 female F344/N rats and B6C3F$_1$ mice 6 hours per day, 5 days per week, for 103 weeks. The exposure concentrations used (0, 200, or 400 ppm for rats and 0, 100, or 200 ppm for mice) were selected on the basis of results from 13-week inhalation studies in which groups of 10 rats and 10 mice of each sex were exposed to tetrachloroethylene at 100-1,600 ppm for 6 hours per day, 5 days per week.

During the 13-week studies, 1,600 ppm tetrachloroethylene was lethal to 20%-70% of the rats and mice and reduced the final body weights of survivors. In rats, tetrachloroethylene at 200-800 ppm caused minimal to mild hepatic congestion. In dosed male and female mice, minimal to mild hepatic leukocytic infiltration, centrilobular necrosis, bile stasis (400-1,600 ppm), and mitotic alteration (200-1,600 ppm) were produced. Tetrachloroethylene exposure also caused minimal renal tubular cell karyomegaly in mice at concentrations as low as 200 ppm.

During the 2-year studies, exposure to tetrachloroethylene did not consistently affect body weight gains in either rats or mice. Exposure at 400 ppm tetrachloroethylene reduced the survival of male rats (control, 23/50; low dose, 20/50; high dose, 12/50). This reduced survival may have been related to an increased incidence of mononuclear cell leukemia. Tetrachloroethylene at both exposure concentrations reduced the survival of male mice (46/50; 25/50; 32/50), whereas exposure at 200 ppm reduced female mouse survival (36/50; 31/50; 19/50). Early deaths in mice may have been related to the development of hepatocellular carcinomas.

Both concentrations of tetrachloroethylene were associated with increased incidences of mononuclear cell leukemia in male rats (28/50; 37/50; 37/50). In female rats, tetrachloroethylene increased the incidence of leukemia (18/50; 30/50; 29/50) and decreased the time to occurrence of the disease. Tetrachloroethylene produced renal tubular cell karyomegaly in male and female rats, renal tubular cell hyperplasia in male rats, and renal tubular cell adenomas or adenocarcinomas (combined) in male rats (1/49; 3/49; 4/50).

The incidence of the renal tubular cell tumors was statistically significant; these uncommon tumors have been consistently found at low incidences in male rats in other 2-year studies of chlorinated ethanes and ethylenes. One low dose male rat had a kidney lipoma, and another had a nephroblastoma. Four high dose male and two high dose female rats had gliomas of the brain, whereas one control male and one control female had this tumor.

In male and female mice, tetrachloroethylene caused dose-related increases in the incidences of hepatocellular neoplasms. In males, tetrachloroethylene at 200 ppm increased the incidence of hepatocellular adenomas (11/49; 8/49; 18/50) and at both concentrations increased the incidence of hepatocellular carcinomas (7/49; 25/49; 26/50). In female mice, tetrachloroethylene at both concentrations increased the incidences of hepatocellular carcinomas (1/48; 13/50; 36/50). Tetrachloroethylene also produced renal tubular cell karyomegaly in both sexes of mice, and one low dose male mouse had a tubular cell adenocarcinoma.

In these inhalation studies, there were no neoplastic changes in the respiratory tracts of either species, but there was an increase in the incidence of squamous metaplasia in the nasal cavities in dosed male rats (0/50; 5/50; 5/50).

Tetrachloroethylene was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of male Syrian hamster or male Sprague-Dawley rat liver S9. Tetrachloroethylene was not mutagenic in L5178Y/TK$^{+/-}$ mouse lymphoma cells with or without metabolic activation and did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*. Tetrachloroethylene did not induce sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of metabolic activation.

An audit of the experimental data was conducted for these 2-year studies on tetrachloroethylene. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenicity* of tetrachloroethylene for male F344/N rats as shown by an increased incidence of mononuclear cell leukemia and uncommon renal tubular cell neoplasms. There was *some evidence of carcinogenicity* of tetrachloroethylene for female F344/N rats as shown by increased incidences of mononuclear cell leukemia. There was *clear evidence of carcinogenicity* for B6C3F$_1$ mice as shown by increased incidences of both hepatocellular adenomas and carcinomas in males and of hepatocellular carcinomas in females.

Synonyms: carbon bichloride; carbon dichloride; ethylene tetrachloride; per; perc; perchlor; perchlorethylene; perchloroethylene; perk; tetrachlorethylene; 1,1,2,2-tetrachloroethylene

Trade names: Ankilostin; Antisal 1; Dee-Solv; Didakene; Dow-Per; ENT 1860; Fedel-Un; Nema; Perclene; Percosolv; Perklone; PerSec; Tetlen; Tetracap; Tetraleno; Tetravec; Tetroguer; Tetropil

Report Date: August 1986

Note: Tetrachloroethylene was previously tested in Osborne-Mendel rats and B6C3F$_1$ mice by gavage (See TR-13, reported 1977).

TR-312 Toxicology and Carcinogenesis Studies of *n*-Butyl Chloride (CAS No. 109-69-3) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of *n*-butyl chloride (greater than 99.5% pure), a solvent as well as an alkylating agent, were conducted by exposing groups of F344/N rats and B6C3F$_1$ mice to *n*-butyl chloride in corn oil by gavage for 14 days, 13 weeks, and 2 years. In the 14-day studies, no compound-related gross pathologic effects were observed in groups of five male or female rats or mice administered doses of up to 3,000 mg/kg body weight. However, deaths occurred in the groups administered 750, 1,500, or 3,000 mg/kg. Tremors and convulsions following gavage administration were observed.

In the 13-week studies, groups of 10 male and 10 female rats were administered up to 500 mg/kg *n*-butyl chloride, and similar groups of mice received up to 1,000 mg/kg, Three of 10 male rats in the 500 mg/kg dose group and one female mouse in the 120 mg/kg dose group died before the end of the studies. Mild to moderate extramedullary hematopoiesis was observed in 3/10 male rats receiving 500 mg/kg. Mean body weights of male and female rats receiving 250 or 500 mg/kg were lower than those of the vehicle controls. Convulsions were observed in male and female rats receiving 250 mg/kg or higher and in 2/10 female mice receiving 1,000 mg/kg. Based on these results, 2-year toxicology and carcinogenesis studies of *n*-butyl chloride were conducted by administering doses of 0, 60, or 120 mg/kg in corn oil by gavage to groups of 50 male and 50 female rats and doses of 0, 500, or 1,000 mg/kg to groups of 50 male and 50 female mice.

In the 2-year studies, survival relative to that of vehicle controls was significantly lower in high dose male rats (40/50 vs 17/50) and high dose female rats (35/50 vs 11/50) and in male mice receiving 1,000 mg/kg (33/50 vs 10/50). Due to excessive mortality in the 1,000 mg/kg female mice, the group was terminated in the 45th week and a second series of 2-year studies in mice of each sex was started at concentrations of 0 and 250 mg/kg. Male mice in the 1,000 mg/kg group had 10% lower mean body weights than the vehicle control group. No adverse effects on survival or body weights in other dosed groups of rats and mice were observed. Convulsions were observed before or after gavage administration on several occasions during the rat studies. These observations were noted primarily in the high dose groups (male: vehicle control, 1/50; low dose, 3/50; high dose, 27/50; female: vehicle control, 0/50; low dose, 7/50; high dose, 45/50). Hemorrhage of the brain and alveoli were observed primarily in high dose male and female rats dying from convulsions. Lymphoid depletion of the spleen and splenic hemosiderosis were also observed in these animals. In mice, convulsions were observed only in the first studies (in the high dose female mice that were terminated early and in 6/50 high dose male mice).

Pheochromocytomas of the adrenal gland occurred at marginally increased incidence in low dose female rats (1/50; 6/50; 1/49). Hyperplasia was observed in 3/50 vehicle controls, 7/50 low dose females, and 4/49 high dose females. The incidence of pheochromocytomas was low, not dose related, and not seen in males, and thus it was not considered to be compound related. Cytoplasmic vacuolization of the adrenal cortex occurred at increased incidences in males (5/50; 10/50; 20/50) but not in female rats. Nephropathy of the kidney occurred at increased incidences in female rats (13/50; 25/50; 20/50) but not in male rats. Additional nonneoplastic lesions such as congestion, inflammation, or nephrosis were not present to any degree in either vehicle control or dosed female rats.

An increased incidence of alveolar/bronchiolar adenomas or carcinomas (combined) was observed in the 500 mg/kg group of female mice (3/50 vs 9/50), but little effect was seen in the 250 mg/kg group (6/50 vs 8/50). The incidences of adenomas or carcinomas (combined) in dosed female mice were not significantly different from that in the pooled vehicle control group from the first and second studies (pooled controls, 9/100; 250 mg/kg, 8/50; 500 mg/kg, 9/50). The lack of hyperplasia in female mice and the negative trend in male mice suggest that these marginal effects were probably not related to the administration of *n*-butyl chloride.

An increased incidence of hepatocellular adenomas or carcinomas (combined) was observed in the 500 mg/kg dose group of female mice (3/50 vs 8/50) but not in the 250 mg/kg dose group (9/50 vs 7/50). An increased incidence of hemangiosarcomas was observed in male mice in the first study (1/50; 3/50; 4/50) but not in the second study (4/50 vs 2/50). Neither of these marginal effects was regarded as compound related.

n-Butyl chloride was not mutagenic in *Salmonella typhimurium* strains TA98, TA1535, or TA1537 in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 or in the presence of male Syrian hamster liver S9. *n*-Butyl chloride was mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay in the absence of Aroclor-induced male rat liver S9 and was not tested in the presence of S9. *n*-Butyl chloride did not induce sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of Aroclor-induced male rat liver S9.

An audit of the experimental data was conducted for the 2-year studies of *n*-butyl chloride. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenicity* of *n*-butyl chloride for male and female F344/N rats at daily doses of 60 or 120 mg/kg, for male B6C3F$_1$ mice at doses of 250, 500, or 1,000 mg/kg, or for female B6C3F$_1$ mice at doses of 250 or 500 mg/kg. Chemical-induced toxicity in high

dose rats (primarily females) reduced the sensitivity of the study for determining carcinogenicity.

Synonyms: 1-chlorobutane; butyl chloride; *n*-propylcarbinyl chloride

Report Date: April 1986

TR-313 Toxicology and Carcinogenesis Studies of Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-Dodecachlorooctahydro-1,3,4-metheno-1*H*-cyclobuta[*cd*]pentalene) (CAS No. 2385-85-5) in F344/N Rats (Feed Studies)

Mirex (95% pure), formerly used a systemic insecticide and as a fire retardant, was studied for toxicologic and carcinogenic effects by administering diets containing 0, 0.1, 1.0, 10, 25, or 50 ppm mirex to groups of 52 F344/N rats of each sex for 104 weeks. Doses selected for the 2-year studies were based primarily on the effects on body weights and survival of rats in a 26-week study. During the first 6 months of the 2-year study, because of good survival and the absence of observable toxic effects in female rats, additional groups (termed second study) of 52 F344/N female rats were started at higher dietary concentrations of 0, 50, and 100 ppm mirex. Based on feed consumption data, the estimated average intake per day was 0, 0.007, 0.075, 0.75, 1.95, and 3.85 mg mirex/kg body weight for male rats and female rats in the first study, and 0, 3.9, and 7.7 mg/kg for female rats in the additional study.

Body Weights, Feed Consumption, and Survival in Two-Year Studies: Mean body weights of male rats that received 25 or 50 ppm mirex were 5%-18% lower than those of the controls throughout most of the study; mean body weights of female rats that received 50 or 100 ppm mirex were 4%-18% lower than those of the controls after week 40; mean body weights of groups receiving 0.1, 1.0, or 10 ppm were similar to those of controls. Feed consumption by dosed male rats was 83%-91% that by controls, and that by dosed female rats was 86%-99% that by controls. The top dietary exposure groups of rats received the equivalent of 3.85 mg mirex/kg body weight, whereas the 100-ppm group of female rats (second study) averaged 7.7 mg/kg. At the end of the study, survival of male rats that received 25 or 50 ppm of mirex was lower than that of controls, whereas survival of all dosed groups of female rats was similar to that of controls (male: control, 44/52; 0.1 ppm, 37/52; 1 ppm, 36/52; 10 ppm, 37/52; 25 ppm, 19/52; 50 ppm, 15/52; female – first study: 38/52; 38/52; 35/52; 41/52; 35/52; female – second study: control, 44/52; 50 ppm, 44/52; 100 ppm, 39/52).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The most notable compound-related effects were observed in the liver of male and female rats. Fatty metamorphosis, cytomegaly, angiectasis (males only), and necrosis of the liver were observed at increased incidences in dosed rats. The incidences of of neoplastic nodules of the liver were dose related, and in the 10-, 25-, and 50-ppm groups of males and the 50- and 100-ppm groups of females (second study), they were markedly greater than those in controls (52/group – male: control, 3; 0.1 ppm, 5; 1 ppm, 5; 10 ppm, 14; 25 ppm, 15; 50 ppm, 26; female (second study): control, 2; 50 ppm, 23; 100 ppm, 30). In the first study in female rats, the incidences of neoplastic nodules were not significantly different between control and dosed groups (10; 5; 4; 5; 9; 7). The 10 neoplastic nodules of the liver seen in the control group (19%) was significantly greater than the mean incidence observed historically (57/2,015; 2.8%). The incidences of hepatocellular carcinomas in control and dosed groups were relatively low and were not significantly different between groups.

The incidences of pheochromocytomas of the adrenal gland occurred with a positive trend in male rats (8/51; 7/52; 13/52; 11/52; 18/51, 19/51); the incidences in the 25- and 50-ppm male rats were greater than that in controls; malignant pheochromocytomas were observed in 2 controls and in 2 mirex-exposed male rats. The incidence of pheochromocytomas in 50-ppm female rats in the first study was marginally greater than that in controls (control, 1/51; 50 ppm, 6/52); this borderline increase was not observed in the second female rat study and thus is not considered to be due to the dietary administration of mirex.

Nephropathy occurred at similar incidences in control and mirex-exposed groups of male and female rats; however, the severity of this nonneoplastic lesion was judged to be slightly greater in the groups given 25, 50, or 100 ppm mirex (male: severe vs. moderate in controls; female: moderate to severe vs. moderate). Hyperplasia of the transitional epithelium of the kidney pelvis was observed in dosed male rats (0/51; 2/51; 2/52; 5/52; 14/51; 9/52). Transitional cell papillomas of the renal pelvis in male rats occurred with a positive trend ($P<0.02$) (0/51; 0/51; 0/52; 1/51; 3/52). The highest incidence previously observed in untreated male F344/N rats in NTP studies is 1/48, and the mean historical incidence is 5/1,968 (0.3%).

In both the first and second studies in female rats, the incidence of mononuclear cell leukemia showed dosed-related increases (first study: 8/52; 8/52; 11/52; 14/52; 18/52; 18/52; second study: 6/52; 9/52; 14/52). When the data from both studies are combined, the incidences are significantly increased in the 10-, 25-, 50-, and 100-ppm groups. The mean historical incidence is 19% (375/2,021).

For the thyroid gland, there was a positive trend for follicular cell neoplasms in male rats (0/51; 1/50; 0/47; 1/47; 0/35; 4/49) and a negative trend for C-cell neoplasms in male rats (8/51; 6/50; 4/47; 7/47; 3/35; 0/49) and in female rats in the first study (12/50; 13/50; 7/48; 9/47; 6/48; 2/46). Neither observation is considered to be associated with the dietary administration of mirex.

Genetic Toxicology: Mirex was not mutagenic in the *Salmonella typhimurium*-microsome assay when tested in a preincubation protocol in the presence or absence of exogenous metabolic activation in strains TA98, TA100, TA1535, or TA1537. Mirex did not induce either sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of S9.

Conclusions: Under the conditions of these 2-year feed studies of mirex, there is *clear evidence of carcinogenic activity* for male and female F344/N rats, as primarily indicated by marked increased incidences of benign neoplastic nodules of the liver, as well as by increased incidences of pheochromocytomas of the adrenal gland and transitional cell papillomas of the kidney in males and by increased incidences of mononuclear cell leukemia in females.

Nonneoplastic effects induced by mirex include cytomegaly, fatty metamorphosis, angiectasis (males only), and cellular necrosis in the liver.

Synonyms and Trade Names: 1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1*H*-cyclobuta [*cd*]pentalene; hexachloropentadiene dimer; dodecachloropentacyclodecane; perchloropentacyclodecane; hexachlorocyclopentadiene dimer; Dechlorane®; Ferriamicide®

Report Date: February 1990

TR-314 Toxicology and Carcinogenesis Studies of Methyl Methacrylate (CAS No. 80-62-6) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

Toxicology and carcinogenesis studies of methyl methacrylate, a liquid chemical intermediate used in the plastics industry in the manufacture of plexiglass and other acrylic products, were conducted by exposing groups of F344/N rats and B6C3F$_1$ mice by inhalation for 14 weeks and 2 years.

In the 14-week studies, groups of 10 male and 10 female rats and mice were exposed to methyl methacrylate at concentrations of up to 5,000 ppm. All male and female rats and eight male and eight female mice exposed at 5,000 ppm died, one male and nine female rats and four male and no female mice exposed at 3,000 ppm died, and one male and three female rats and two male and one female mouse exposed at 2,000 ppm died; all rats and mice exposed at 500 or 1,000 ppm survived. Compared with the controls, the body weights of the exposed male and female rats and mice were lower. Compound-related lesions included inflammation associated with necrosis and loss of olfactory epithelium in the nasal turbinates in both male and female rats; malacia and gliosis in female rats; inflammation of the nasal turbinates and nasal epithelium metaplasia in both male and female mice; and renal cortical necrosis, renal cortical tubular degeneration, renal focal mineralization, and liver necrosis in male mice. Based on these results, 2-year inhalation toxicology and carcinogenesis studies were conducted in which groups of 50 male rats were exposed to methyl methacrylate at 0, 500, or 1,000 ppm; female rats at 0, 250, or 500 ppm; and male and female mice at 0, 500, or 1,000 ppm.

In the 2-year studies, the body weights of the low dose and high dose male and female rats were within 10% of those of the controls. There was no difference in survival between the dosed male and female rats and the controls. Incidences of inflammation of the nasal cavity and degeneration of the olfactory sensory epithelium were greater in the dosed male and female rats than in the controls, with lesions seen in virtually all high dose animals.

An increased incidence of mononuclear cell leukemia was observed in female rats exposed to methyl methacrylate at 500 ppm compared with the controls (control, 11/50; 250 ppm, 13/50; 500 ppm, 20/50). This increase was not significant by life table tests, the method of analysis most appropriate for this fatal neoplasm.

The mean body weights of dosed male and female mice were 5%-8% lower than those of the controls at the end of the 2-year studies. However, during most of the second year of the studies, body weights of dosed male mice and high dose female mice were 10%-18% lower than those of the controls. Survival rates of the dosed and control mice were similar.

Incidences of inflammation and epithelial hyperplasia of the nasal cavity and degeneration of the olfactory sensory epithelium were significantly greater in all dosed groups of male and female mice compared with those of the controls. Compound-related neoplastic lesions were not found in the dosed mice.

Significant dose-related decreases were observed in the incidences of pituitary gland and preputial gland tumors in male rats, alveolar/bronchiolar adenomas or carcinomas (combined) in male mice, hepatocellular adenomas in both male and female mice, and pituitary gland adenomas or adenocarcinomas (combined) and uterine adenocarcinomas in female mice.

Methyl methacrylate was not mutagenic in strains TA100, TA1535, TA97, or TA98 of *Salmonella typhimurium* in the presence or absence of male rat or hamster liver S9 when assayed by a preincubational protocol but gave a positive response in L5178Y/TK$^{+/-}$ mouse lymphoma cells in the presence or absence of male rat liver S9. In cultured Chinese hamster ovary cells, methyl methacrylate produced a reproducible, dose-related increase in the frequency of sister-chromatid exchanges, both with and without rat liver S9. A slight, dose-related increase in chromosomal aberrations was also induced in cultured Chinese hamster ovary cells in the absence of S9; in the presence of S9, an increase in the frequency of aberrations was seen only at the highest, near-lethal dose of 5 mg/ml.

An audit of the experimental data was conducted for the 2-year carcinogenesis studies on methyl methacrylate. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenicity* of methyl methacrylate for male F344/N rats exposed at 500 or 1,000 ppm, for female F344/N rats exposed at 250 or 500 ppm, or for male and female B6C3F$_1$ mice exposed at 500 or 1,000 ppm. Inhalation of methyl methacrylate was associated with inflammation of the nasal cavity and degeneration of the olfactory sensory epithelium in male

and female rats and mice; epithelial hyperplasia of the nasal cavity was also observed in exposed mice.

Synonyms: acrylic acid, 2-methyl, methyl ester; methacrylic acid, methyl ester; methyl α-methylacrylate; methyl methylacrylate; methyl-2-methylpropenoate; methyl-2-methyl-2-propenoate; 2-methyl-2-propenoic acid methyl ester; MME

Report Date: October 1986

TR-315 Toxicology and Carcinogenesis Studies of Oxytetracycline Hydrochloride (CAS No. 2058-46-0) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Toxicology and carcinogenesis studies were conducted on oxytetracycline hydrochloride (greater than 98.8% pure), a broad-spectrum antibiotic. Groups of F344/N rats and B6C3F$_1$ mice were fed diets containing oxytetracycline hydrochloride for a series of 14-day, 13-week, and 2-year studies. In the 14-day studies, no compound-related gross pathologic effects were seen in rats or mice (groups of five animals per sex per species) given up to 100,000 ppm in their feed. The final mean body weight of male rats receiving in feed was 27% lower than that of the controls. Final mean body weights of mice that received 25,000, 50,000, or 100,000 ppm were lower (male: 11%; 16%; 17%; female: 6%; 5%; 17%) than those of the controls. In the 13-week studies, groups of 10 male and 10 female rats and mice were fed diets containing up to 50,000 ppm in feed, and no chemically related gross or histopathologic effects were observed in mice of either sex or in female rats. In male rats, fatty metamorphosis of minimal severity was diagnosed in the liver of 5/10 animals at 6,300, 12,500, and 50,000 ppm and in 2/10 animals at 3,100 and 25,000 ppm. None was seen in the controls. Oxytetracycline levels in bones of rats and mice (as determined fluorometrically) at the end of the 13-week studies increased with dose, the highest levels (3-10 times background levels) being observed at 50,000 ppm.

The 2-year toxicology and carcinogenesis studies were conducted by administering diets containing 0, 25,000, or 50,000 ppm oxytetracycline hydrochloride to groups of 50 male and 50 female rats and diets containing 0, 6,300, or 12,500 ppm oxytetracycline hydrochloride to groups of 50 male and 50 female mice for 103 weeks. The highest dose selected for rats was considered to be the maximum level that would not affect the nutritional value of dosed feed. The dietary concentrations correspond to the following approximate doses: rats—0, 1,000, or 2,000 mg/kg body weight per day; mice—0, 650, or 1,400 mg/kg per day.

Mean body weights were approximately 5%-8% lower than those of controls in high dose male rats during weeks 4-47, in high dose male mice after week 31, and in high dose female mice after week 26. The mean body weights of dosed female rats and low dose male and female mice were comparable to those of controls. The survival of control male rats was lower than that of the high dose group (22/50 vs 38/50). No significant differences in survival were observed between the remaining groups of rats or between any groups of mice.

Pheochromocytomas of the adrenal gland occurred with positive trends in male rats (control, 10/50; low dose, 18/50; high dose, 24/50), and the incidence in the high dose group was greater than that in the controls. Two additional control males and one additional low dose male had malignant pheochromocytomas. The incidence of adrenal gland medullary hyperplasia was elevated slightly but not significantly in dosed male rats (7/50; 14/50; 9/50).

Adenomas and adenomas and adenocarcinomas (combined) of the pituitary gland in female rats occurred with positive trends, and the incidences in the high dose group were greater than that in the controls (adenomas: 19/50; 17/50; 30/50; adenomas or adenocarcinomas [combined]: 20/50; 24/50; 32/50). The incidence of pituitary gland hyperplasia was slightly decreased in dosed female rats (16/50; 10/50; 11/50).

No compound-related increases in nonneoplastic or neoplastic lesions were observed in male or female mice.

Oxytetracycline hydrochloride was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9 when assayed according to the preincubational protocol. Oxytetracycline hydrochloride was mutagenic in L5178Y/TK$^{+/-}$ mouse lymphoma cells in the presence but not in the absence of Aroclor 1254-induced male rat liver S9. In cultured Chinese hamster ovary cells, oxytetracycline was weakly positive in inducing sister-chromatid exchanges both with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9 but did not induce chromosomal aberrations.

An audit of the experimental data was conducted for these 2-year carcinogenesis studies of oxytetracycline hydrochloride. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year feed studies of oxytetracycline hydrochloride, there was *equivocal evidence of carcinogenicity* for male F344/N rats, as indicated by increased incidences of pheochromocytomas of the adrenal gland. There was *equivocal evidence of carcinogenicity* for female F344/N rats fed diets containing oxytetracycline hydrochloride, as indicated by increased incidences of adenomas of the pituitary gland. There was no evidence of carcinogenicity for male or female B6C3F$_1$ mice fed diets containing 6,300 or 12,500 ppm oxytetracycline hydrochloride for 2 years.

Synonyms: 2-naphthacenecarboxamide,4(dimethylamino)-1, 4,4a,5,5a,6,11,12a-octahydro-3,6-10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-monohydrochloride; Biosolvmycin; Hydrocyclin; Liquamycin; Otetryn; Oxlopar; 5-hydroxytetracycline hydrochloride; Terramycin Hydrochloride; Tetramine; Tetran Hydrochloride

Report Date: January 1987

TR-316 Toxicology and Carcinogenesis Studies of Dimethylvinyl Chloride (1-Chloro-2-Methylpropene) (CAS No. 513-37-1) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Dimethylvinyl chloride is a clear colorless liquid, which, because of its volatility and flammability at room temperature, is a significant fire hazard. It has a boiling point of 68.1° C (155° F) and a density at 20° C of 0.919 g/ml. Dimethylvinyl chloride is a byproduct in the production of 3-chloro-2-methylpropene by the chlorination of isobutene. It is not known to be produced in the United States for other than laboratory purposes. This chemical was nominated for toxicologic studies because of its reported presence in ambient air in the Baltimore area and was selected for toxicologic characterization because of its structural similarity to the known animal and human carcinogen, vinyl chloride monomer.

Toxicology and carcinogenesis studies of dimethylvinyl chloride (96%-98% pure), a structural analog of vinyl chloride monomer, a known human carcinogen, by administered dimethylvinyl chloride in corn oil by gavage to groups of 50 male and 50 female F344/N rats and B6C3F$_1$ mice at doses of 0, 100, or 200 mg/kg body weight 5 days per week for 102 or 103 weeks. The selection of these doses was based on results of 13-week studies, which included depression of body weight at doses of 500 mg/kg or above in rats as well as histopathologic changes intestinal epithelium, bone marrow, hepatocytes, and the testes at doses of 250 mg/kg and above; doses in mice were selected on the basis of histopathologic changes in lymphopoietic cells, liver, pancreatic islets, ovary, testis, and spleen, with changes being most prominent at doses of 500 mg/kg and above.

In the 2-year studies, body weights of rats and mice given 100 mg/kg were comparable to those of the vehicle controls except for the last few weeks in mice when body weights were markedly lower than those for the vehicle controls. At 200 mg/kg, the mean body weights of rats and mice were progressively decreased relative to those of vehicle controls, with the significant departure from vehicle controls occurring somewhat earlier in males than in females. Survival of vehicle control rats and mice was comparable to historical values; however, survival of dosed male and female rats was significantly lower than that of vehicle controls, with the incidence of mortality being more severe at the high dose than at the low dose. There were no survivors in the high dose group of male rats after week 85 or in the high dose group of female rats after week 97. Survival was significantly lower among dosed male and female mice compared with vehicle controls. In the absence of toxicological findings that would explain the early deaths, it is assumed that the high incidence of tumors and chemical- related toxicity contributed to the decreased survival of dosed rats and mice.

In rats, the severity and incidence of nonneoplastic lesions were minimal; these lesions included necrosis of the duodenum and epithelial hyperplasia at the sites of tumor formation – the nasal cavity, esophagus, and forestomach. In mice, the severity of nonneoplastic lesions was also minimal; the lesions included necrosis of the liver, bone marrow granulocytic hyperplasia, and inflammation of the nasal cavity (small number, females only.)

Several types of neoplastic lesions occurred with significantly increased incidences in dosed animals as shown in the following table (see page 11 of Technical Report). Among rats, these lesions included malignant epithelial tumors of the nasal cavity and squamous cell tumors of the oral cavity, esophagus, and forestomach in males and females. The increased number of fibroadenomas of the mammary gland in female rats may have been related to dimethylvinyl chloride administration. The lack of a clear dose-response relationship for certain tumors in rats is considered to be related to the increased number of early deaths observed in the high dose groups.

Among dosed mice, there were significantly increased incidences of squamous cell carcinomas of the forestomach (both sexes), squamous cell papillomas of the forestomach (males), and squamous cell carcinomas of the preputial gland (males). The increased incidence of papillary adenomas of the harderian gland and alveolar/bronchiolar adenomas or carcinomas in female mice may have been related to administration of dimethylvinyl chloride.

Limited metabolism studies of ^{14}C-labeled dimethylvinyl chloride were conducted in male F344/N rats and B6C3F$_1$ mice. Single doses of 150 mg/kg were administered to rats for 1, 2, or 4 consecutive days. About 25% of the administered doses was exhaled as carbon dioxide; this amount was independent of the number of doses administered. Another 25%-35% of the administered dose was exhaled; 96% of this parent was material. Approximately 35% and 6% were excreted in the urine and feces, respectively. The elimination half-life of radioactive label was 3-4 days for the liver and kidney, the two organs containing the greatest amounts of the administered dose. In mice, a much smaller fraction of the dose was exhaled and a larger proportion was excreted in urine compared with rats.

Dimethylvinyl chloride was not mutagenic in four strains of *Salmonella typhimurium* with or without metabolic activation, but it was mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay in the absence of metabolic activation. Sister-chromatid exchanges were induced in Chinese hamster ovary cells with and without metabolic activation, but there was no increase in chromosomal aberrations. When fed to Drosophila, dimethylvinyl chloride induced significant increases in the frequencies of both sex-linked recessive lethal mutations and reciprocal translocations.

Studies of the immunotoxicity of dimethylvinyl chloride were conducted in which female B6C3F$_1$ mice received daily oral doses of 0, 50, 100, 200, or 400 mg dimethylvinyl chloride per kilogram body weight. Compound-related increases in susceptibility to bacterial infection and decreases in macrophage cytostasis were observed at all doses. At the highest dose, the decreased resistance to bacterial and viral challenge

could be related to alterations in specific immune function. However, the increased mortality in rats and mice in the 2-year studies was not relatable to infectious processes.

An audit of the experimental data was conducted for these 2-year toxicology and carcinogenesis studies on dimethylvinyl chloride. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenicity* of dimethylvinyl chloride for both sexes of F344/N rats and $B6C3F_1$ mice. This was based on increased incidences of neoplasms of the nasal cavity, oral cavity, esophagus, and forestomach of male and female F344/N rats. $B6C3F_1$ mice showed increased incidences of squamous cell neoplasms of the forestomach in males and females and squamous cell carcinomas of the preputial gland in males.

Synonym: 1-chloro-2-methylpropene

Report Date: August 1986

TR-317 Toxicology and Carcinogenesis Studies of Chlorpheniramine Maleate (CAS No. 113-92-8) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of chlorpheniramine maleate (99% pure), a widely used antihistaminic drug in human and veterinary medicine, were conducted by administering this chemical in deionized water by gavage to groups of 50 male and 50 female F344/N rats and $B6C3F_1$ mice, 5 days per week for 103 weeks. The doses used were: male rats – 0, 15, or 30 mg/kg; female rats – 0, 30, or 60 mg/kg; male mice – 0, 25, or 50 mg/kg; female mice – 0, 100, or 200 mg/kg. The selection of these doses was based largely on data from 14-day or 16-day studies and 13-week studies in which reduced body weight gain and reduced survival occurred at higher doses. Doses used in the 2-week studies ranged from 40 to 640 mg/kg in rats and 25 to 800 mg/kg in mice; in the 13-week studies, doses ranged from 3.75 to 60 mg/kg in rats and 12.5 to 200 mg/kg in mice. The recommended human adult daily oral dose of chlorpheniramine maleate is up to 0.32 mg/kg.

Doses originally selected for male mice in the 2-year study were 0, 100, or 200 mg/kg; however, because of poor survival, that study was stopped and a new study was started at doses of 0, 25, or 50 mg/kg. At the termination of the study (week 104), survival of high dose female rats (6/50) and high dose male mice (15/50) was lower than that of the vehicle controls (29/50 and 39/50, respectively). Survival of all other dosed groups was comparable to that of respective vehicle control groups. Mean body weights of dosed rats were about 10%-15% (male) or about 10%-25% (female) lower than those of vehicle controls; mean body weights of female mice were generally 20%-35% lower than those of vehicle controls.

No compound-related gross or microscopic pathologic effects were observed in either species in the 16-day or 13-week studies. Hyperactivity and hyperexcitability associated with dosing were frequently noted in the 13-week and 2-year studies. There were no significant positive trends or increases in the incidences of neoplasms in either male or female rats dosed with chlorpheniramine maleate for 103 weeks. Marginal increases in the incidences of adrenal gland capsule adenomas in male mice (vehicle control, 2/50; low dose, 7/49; high dose, 4/49) were not considered to be compound related, since there was not a corresponding increase in the incidence of adrenal gland capsule hyperplasia (46/50; 33/49; 22/49). A positive trend was seen for subcutaneous tissue tumors in male mice (4/50; 5/49; 8/50); this marginal effect was not considered to be compound related.

The incidences of thyroid gland follicular cell cysts (2/48; 10/49; 13/47), thyroid gland follicular cell hyperplasia (3/48; 29/49; 36/47), and thyroid gland follicular cell adenomas (0/48; 4/49; 2/47) were greater in dosed female mice than in vehicle controls. This finding is toxicologically important, since thyroid gland neoplasms are uncommon in mice and are often preceded by hyperplasia of the follicular epithelium.

The major route of excretion of chlorpheniramine or its metabolites is in the urine. In male F344 rats orally administered ^{14}C-chlorpheniramine maleate at doses of 2 or 20 mg/kg, there was essentially no difference in the percentage of urinary or fecal excretion of radioactivity between these dose levels.

Chlorpheniramine maleate was not mutagenic to Salmonella strains TA98, TA100, TA1535, or T1537 in the presence or absence of S9 metabolic activation systems prepared from the liver of Aroclor 1254-treated male Sprague-Dawley rats or male Syrian hamsters. Chlorpheniramine maleate did not induce forward mutations at the TK locus of L5178Y mouse lymphoma cells with or without metabolic activation. In Chinese hamster ovary cells in culture, chlorpheniramine maleate induced a weak but reproducible increase in sister-chromatid exchanges in the absence of exogenous metabolic activation. Chromosomal aberrations were induced at the highest dose tested but only in the presence of S9 from Aroclor 1254-induced Sprague-Dawley male rat liver.

An audit of the experimental data was conducted for these 2-year carcinogenesis studies on chlorpheniramine maleate. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenicity* for F344/N rats or $B6C3F_1$ mice of either sex administered chlorpheniramine maleate in deionized water, 5 days per week for 2 years. Due to high mortality in high dose female rats and high dose male mice, the sensitivity of these groups to detect a carcinogenic response was reduced. Chlorpheniramine maleate had a proliferative effect in the thyroid gland of female mice, as shown by the increased incidences of follicular cell cysts and hyperplasia in both low dose and high dose groups.

Synonyms: 2-*p*-chloro-α-(2-dimethylaminoethyl) benzyl]pyridine maleate; 2-Pyridinepropanamine; γ-[4-chlorophenyl]-N,N-dimethyl-[z]-2-butenedioate

Trade Names: Allerclor; Allergisan; Antagonate; Chlormene; Chlorprophenpyridamine maleate; Chlor-Trimeton; Chlor-Tripolon; Chloropiril; C-Meton; Histadur; Histaspan; Lorphen; M.P. Chlorcaps T.D.; Piriton; Pyridamal-100; Teldrin

Report Date: September 1986

TR-318 Toxicology and Carcinogenesis Studies of Ampicillin Trihydrate (CAS No. 7177-48-2) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Ampicillin trihydrate is a broad-spectrum semisynthetic penicillin that is effective in the treatment of gram-positive and gram-negative bacterial infections produced by Streptococcus, *Bacillus anthracis*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Escherichia coli*. This antibiotic is used in the treatment of upper respiratory tract infections, genital and urinary tract infections, and otitis media in children.

Toxicology and carcinogenesis studies of ampicillin trihydrate (97%-99% pure) were conducted by administering the chemical in corn oil by gavage to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex, 5 days per week for 103 weeks. Male and female rats received doses of 0, 750, or 1,500 mg/kg, and male and female mice received doses of 0, 1,500, or 3,000 mg/kg. Doses selected for the 2-year studies were based on the lack of body weight effects and histopathologic effects at 2,400 mg/kg in the 14-day studies and 3,000 mg/kg in the 13-week studies. Clinical signs in the 13-week studies included diarrhea at 3,000 mg/kg in male and female rats and male mice. Corn oil suspensions containing more than 300 mg ampicillin trihydrate/ml were too viscous to be administered by gavage; therefore, a high dose of 1,500 mg/kg was selected for rats and a high dose of 3,000 mg/kg was selected for mice.

During the 2-year studies, mean body weights of male and female rats were similar to or slightly increased over those of the corresponding vehicle control groups. Mean body weights of low dose and high dose male mice were similar to those of the corresponding vehicle group during year 1 of the study but were slightly below those of the vehicle control group during the last half of the study. Mean body weights of low dose and high dose female mice were greater than those of the vehicle controls throughout most of the study. No significant differences in survival were observed in groups of rats or mice of either sex. Clinical signs observed in dosed rats included diarrhea, excessive urination, and chromodacryorrhea and in dosed mice included increased salivation and decreased activity.

In male rats, administration of ampicillin trihydrate was associated with an increased incidence of mononuclear cell leukemia (vehicle control, 5/50; low dose, 14/50; high dose, 13/50). Malignant lymphomas were observed in one additional vehicle control male rat and two low dose male rats. Lymphocytic leukemia was seen in one high dose rat. High dose male rats showed increased incidences of pheochromocytomas of the adrenal gland medulla (13/50; 12/50; 23/49). Malignant pheochromocytomas were observed in 1/50 vehicle control, 5/50 low dose, and 1/49 high dose male rats. The incidence of adrenal gland medullary hyperplasia was not increased in male rats (14/50; 10/50; 8/49). There were increased incidences of C-cell hyperplasia of the thyroid gland in low dose male and high dose female rats. High dose male rats showed increased incidences of hyperkeratosis and acanthosis of the forestomach.

In male and female mice, ampicillin trihydrate administration was associated with increased incidences of forestomach lesions, including ulcers, inflammation, hyperkeratosis, acanthosis, and evidence of fungal infection.

Ampicillin trihydrate was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of Aroclor 1254-induced male Syrian hamster or male Sprague-Dawley rat liver S9 when tested according to preincubation protocol. Ampicillin trihydrate was not mutagenic in L5178Y mouse lymphoma cells with or without metabolic activation. Ampicillin trihydrate did not cause chromosomal aberrations or sister-chromatid exchanges in Chinese hamster ovary cells with or without metabolic activation.

An audit was conducted for these 2-year studies. Animal/carcass identification discrepancies were observed in rats and mice. The most common findings were the failure to clip some toes in rats and opened ear holes in mice. A review of the inlife data (including body weights, clinical observations, and dosing records) indicated that animals had not been interchanged among groups. The data are considered adequate to support the conclusions.

Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenicity* of ampicillin trihydrate for male F344/N rats as shown by increased incidences of pheochromocytomas of the adrenal medulla and by marginally increased incidences of mononuclear cell leukemia. There was *no evidence of carcinogenicity* for female F344/N rats receiving 750 or 1,500 mg/kg or for male and female B6C3F$_1$ mice receiving 1,500 or 3,000 mg/kg per day. Nonneoplastic lesions of the forestomach were seen in male rats and male and female mice.

Synonyms and trade names: Acillin; Amcap; Amcill; Aminobenzylpencillin trihydrate; α-Aminobenzylpencillin trihydrate; Amperil; Ampichel; Ampikel; Ampinova; Amplin; Cymbi; Divercillin; Liffampil; Morepen; Pen A; Pensyn; Polycillin; Princillin; Principen; Ro-ampen; Trafarbiot

Report Date: April 1987

TR-319 Toxicology and Carcinogenesis Studies of 1,4-Dichlorobenzene (CAS No. 106-46-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

1,4-Dichlorobenzene is commonly used as a space deodorant in toilets and for moth control. Because of its extensive production and use and the absence of carcinogenicity data, carcinogenesis studies were conducted by administering 1,4-dichlorobenzene (greater than 99% pure) in corn oil by gavage (5 days per week) to male F344/N rats at doses of 0, 150, or 300 mg/kg and to female F344/N rats and male and female B6C3F$_1$ mice at doses of 0, 300, or 600 mg/kg per day for 2 years (50 animals per group). Fourteen-day and 13-week studies were performed to characterize the toxicity, identify affected sites, and set doses for the 2-year studies. Clinical chemistry and hematologic studies were performed during the 13-week studies to assess the effects of 1,4-dichlorobenzene on the liver, kidney, and hematopoietic system and to assess whether the compound produced hepatic porphyria.

Two 13-week studies were performed in rats. In the first study, rats were dosed with 300-1,500 mg/kg 1,4-dichlorobenzene. Because histologic changes were observed in the kidney of male rats at all doses, a second 13-week study was performed at doses of 38-600 mg/kg. In the 13-week studies, survival was decreased in groups of male rats given 1,200 or 1,500 mg/kg and in female rats given 1,500 mg/kg. Weight gain was decreased in male rats receiving doses of 300 mg/kg or more and in female rats given doses of 1,200 or 1,500 mg/kg. Doses of 1,200 or 1,500 mg/kg produced degeneration and necrosis of hepatocytes, hypoplasia of the bone marrow, lymphoid depletion of the spleen and thymus, and epithelial necrosis of the nasal turbinates in male and female rats. Renal tubular cell degeneration was observed in male rats receiving 300 mg/kg or more in the first study, but only slight changes were seen at 300 mg/kg in the second study. Liver weight to brain weight ratios were increased at 900 mg/kg or more for both male and female rats. The kidney weight to brain weight ratio was increased in male rats receiving doses of 600 mg/kg or more.

Administration of 1,4-dichlorobenzene to rats for 13 weeks produced slight but statistically significant decreases in the hematocrit, red blood cell count, and hemoglobin level in all males receiving doses of 300-1,200 mg/kg. No clear hematologic changes were observed in female rats. 1,4-Dichlorobenzene produced minimal changes in clinical chemistry parameters in the 13-week studies. Serum cholesterol levels were increased by doses of 600 mg/kg or more in male rats and 900 mg/kg or more in female rats. Serum triglycerides were reduced by doses of 300 mg/kg or more in male rats. The blood urea nitrogen level was increased slightly in male rats dosed with 900 mg/kg or more. Urinary porphyrins were increased slightly in male rats administered 1,200 or 1,500 mg/kg and female rats receiving 1,200 mg/kg. However, these increases were modest and indicative of a mild porphyrinuria rather than hepatic porphyria. Liver porphyrins were not increased at any dose.

Two 13-week studies were performed in mice. The doses selected for the first study were 600-1,800 mg/kg. Survival was decreased in male and female mice receiving doses of 1,500 mg/kg or more, and body weight gain was decreased at all doses. Hepatocellular degeneration was observed in both sexes at all doses, and the liver weight to brain weight ratio was increased at doses of 900 mg/kg or more. Serum cholesterol levels were increased in male mice at doses of 900 mg/kg or more, whereas serum protein and triglycerides were increased at doses of 1,500 mg/kg or more. These relatively modest clinical chemistry changes probably reflect the hepatic effects of this compound. The white blood cell count was reduced significantly in male mice receiving doses of 600 mg/kg or more and female mice receiving 1,000 mg/kg or more, but this effect was not dramatic. Hepatic porphyria was not found in mice at any dose in the 13-week study. Because hepatic effects were seen in all dose groups in the first study, a second 13-week study was performed at doses of 85-900 mg/kg. In this study, hepatocellular cytomegaly was observed im male and female mice at doses of 675 mg/kg or more but not at 338 mg/kg. Renal damage was not observed in mice in either 13-week study.

Based on the histopathologic findings in the kidney of male rats and in the liver of both sexes of rats and mice in the 13-week studies, the doses selected for the 2-year studies were 150 and 300 mg/kg for male rats and 300 and 600 mg/kg for female rats and male and female mice. In the 2-year studies, survival of female rats and of both sexes of mice was comparable to that of the vehicle controls; survival of high dose male rats was significantly lower than that of the vehicle controls (vehicle control, 32/50; low dose, 31/50; high dose, 20/50). Mean body weights of high dose male rats were 5%-8% lower than those of vehicle controls after week 38, and those of high dose female rats were 5%-7% lower than those of vehicle controls after week 55. Mean body weights of mice dosed with 1,4-dichlorobenzene were comparable to those of vehicle controls throughout the studies.

Administration of 1,4-dichlorobenzene to male rats increased the average seveity of nephropathy and caused epithelial hyperplasia of the renal pelvis (1/50; 30/50; 31/50), mineralization of the collecting tubules in the renal medulla (4/50; 46/50; 47/50), and focal hyperplasia of renal tubular epithelium (0/50; 1/50; 9/50). There were increased incidences of nephropathy in both low and high dose female rats compared with vehicle controls (21/49; 32/50; 41/49). 1,4-Dichlorobenzene produced a dose-related increase in the incidence of tubular cell adenocarcinomas of the kidney in male rats (1/50; 3/50; 7/50); one tubular cell adenoma was observed in a high dose male rat. These malignant tumors are uncommon in male F344/N rats. They have been diagnosed in only 4/1,098 (0.4%) corn oil gavage controls in previous NTP studies. There were no tubular cell tumors in dosed or vehicle control female rats. There was a marginal increase in the incidence of mononuclear cell leukemia in dosed male rats compared with that in vehicle controls (5/50; 7/50; 11/50).

1,4-Dichlorobenzene increased the incidences of nonneoplastic liver lesions in male and female mice, including alteration in cell size (cytomegaly and karyomegaly), hepatocellular degeneration, and individual cell necrosis. 1,4-Dichlorobenzene also increased the incidences of nephropathy in male mice and renal tubular regeneration in female mice. 1,4-Dichlorobenzene increased the incidences of hepatocellular carcinomas in high dose male (14/50; 11/49; 32/50) and female (5/50; 5/48; 19/50) mice and hepatocellular adenomas in dosed male (5/50; 13/49; 16/50) and high dose female (10/50; 6/48; 21/50) mice. Hepatoblastomas were observed in four high dose male mice but not in vehicle controls. This rare tumor has not occurred in 1,091 male vehicle control mice in NTP studies. An increase in thyroid gland follicular cell hyperplasia was observed in dosed male mice (1/47; 4/48; 10/47), and there was a marginal positive trend in the incidence of follicular cell adenomas of the thyroid gland in female mice (0/48; 0/45; 3/46). Pheochromocytomas (benign or malignant, combined) of the adrenal gland occurred with a positive trend in dosed male mice, and the incidence in the high dose group was significantly greater than in vehicle controls (0/47; 2/48; 4/49). The incidence of adrenal gland medullary hyperplasia in male mice was 2/47; 4/48; and 4/49. Focal hyperplasia of the adrenal gland capsule was also observed in dosed male mice (11/47; 21/48; 28/49).

1,4-Dichlorobenzene was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without activation by Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9 when tested according to a preincubational protocol at concentrations up to 100 μg/plate. 1,4-Dichlorobenzene did not induce forward mutations in the mouse lymphoma L5178Y/TK$^{+/-}$ assay in the absence of exogenous metabolic activation; however, the results were equivocal in this system in the presence of metabolic activation. 1,4-Dichlorobenzene did not produce an increase in sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in culture with or without exogenous metabolic activation. No increase in micronucleated cells was seen in erythrocytes of mice from the first 13-week studies.

An audit of the experimental data was conducted for the 2-year studies of 1,4-dichlorobenzene. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, 1,4-dichlorobenzene produced *clear evidence of carcinogenicity* for male F344/N rats, as shown by an increased incidence of renal tubular cell adenocarcinomas. There was *no evidence of carcinogenicity* for female F344/N rats receiving doses of 300 or 600 mg/kg. There was *clear evidence of carcinogenicity* for both male and female B6C3F$_1$ mice, as shown by increased incidences of hepatocellular carcinomas and hepatocellular adenomas. Marginal increases were observed in the incidences of pheochromocytomas of the adrenal gland in male mice. Nonneoplastic effects in the kidney of male and female rats, in the liver of male and female mice, and in the thyroid gland and adrenal gland of male mice were also associated with the administration of 1,4-dichlorobenzene.

Synonyms: *p*-dichlorobenzene; *para*-dichlorobenzene; *para*-chlorophenyl chloride

Report Date: January 1987

TR-320 Toxicology and Carcinogenesis Studies of Rotenone (CAS No. 83-79-4) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Toxicology and carcinogenesis studies of rotenone (more than 98% pure), a pesticide, were conducted in B6C3F$_1$ mice and F344/N rats for 14 days, 13 weeks, and 2 years.

Results of the Fourteen-Day Studies: In the 14-day studies (dietary rotenone concentrations of 0-600 ppm in the first 14-day studies and 0-4,800 ppm in the second 14-day studies), rough hair coats and dose-related decreases in mean body weight gain were observed in rats. Rats fed diets containing rotenone at concentrations of 1,200 ppm or higher lost weight. No compound-related toxic effects were observed in mice.

Results of the Thirteen-Week Studies: In the 13-week studies (concentrations of 0-1,200 ppm rotenone in feed for rats and 0-50,000 ppm for mice), compound-related effects included lower body weight gain in rats at 150 ppm or more; and bone marrow atrophy and inflammation and hyperplasia of the forestomach in male rats at 300 ppm or more and in female rats at 150 ppm or more. These findings were used to establish the dietary concentrations of rotenone for the 2-year studies.

Experimental Design for the Two-Year Studies: Two-year studies of rotenone were conducted by administering diets containing 0, 38, or 75 ppm rotenone to groups of 50 F344/N rats of each sex for 103 weeks. Groups of 50 B6C3F$_1$ mice of each sex were administered diets containing 0, 600, or 1,200 ppm rotenone on the same schedule. The estimated average amount of rotenone consumed per day was 1.7 mg/kg or 3.5 mg/kg for low dose or high dose rats and 115 mg/kg or 250 mg/kg for low dose and high dose mice.

Survival and Mean Body Weight in the Two-Year Studies: Survival of control and dosed rats was similar (male: control, 22/50; low dose. 31/50; high dose, 30/50; female: control, 27/50; low dose, 32/50; high dose, 31/50). Mean body weights of dosed and control male rats were comparable. Mean body weights of high dose female rats were 5%-9% lower than those of the controls between weeks 58 and 88. Survival of high dose male mice was significantly greater than that of the controls (male: 29/50; 36/50; 47/50; female: 37/50; 42/50; 45/50). Final mean body weights of dosed mice were lower than those of the controls by 8%-13% for males and 17%-24% for females.

Neoplastic Effects in the Two-Year Studies: Parathyroid gland adenomas were observed in 1/41 control, 0/44 low dose, and 4/44 high dose male rats. The historical incidence of this uncommon tumor in untreated control male rats in NTP studies is 4/1,314 (0.3%). Because these tumors are rare and because the highest incidence ever seen in a control group is 1/50, the increase in these tumors may have been related to rotenone administration.

The incidence of subcutaneous tissue fibromas, fibrosarcomas, sarcomas, myxosarcomas, or neurofibrosarcomas (combined) in low dose female rats was greater ($P<0.05$) than that in the controls (0/50; 5/50; 3/50). These tumors were combined because of their possible common histiogenic origin from fibroblasts or undifferentiated mesenchymal cells. The incidence of those tumors in the low dose females was greater than the historical rats at this laboratory (9/337, 3% ± 1%) and throughout the Program (50/2,021, 2% ± 2%). Because of the lack of a significant dose-related trend and because statistical significance was attained only by combining tumors of differing morphology, the subcutaneous tissue tumors in female rats were not considered to be chemically related. The incidences of these tumors in dosed male rats were not significantly different from that in the controls.

Hepatocellular adenomas or carcinomas (combined) occurred in male mice with a negative ($P<0.02$) trend, and the incidence in the high dose group was lower than that in the controls (12/47; 12/49; 1/50). Because this low rate of combined liver tumors is unusual, this decrease may have been related to rotenone administration.

Subcutaneous tissue fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) in male mice occurred with a significant ($P<0.05$) negative trend (8/49; 4/50; 2/50). The incidence in the high dose group was significantly lower than that in the controls by the life table test ($P=0.01$).

Genotoxicity: Rotenone was not mutagenic when tested according to a preincubational protocol with *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with or without metabolic activation by rat or hamster liver S9. Rotenone induced forward mutations in the mouse L5178Y/TK$^{+/-}$ lymphoma assay without activation; it was not tested in the presence of S9. Results of tests with rotenone in Chinese hamster ovary cells were negative for induction of sister chromatid exchanges (SCEs) in the absence of exogenous metabolic activation (at concentrations at which the chemical was very toxic), equivocal for SCEs in the presence of rat liver S9 (due to a nonrepeatable positive response when tests were conducted up to toxic concentrations), and negative for chromosomal aberrations in both the presence and absence of metabolic activation.

Data Audit: An audit of the experimental data was conducted for the 2-year studies of rotenone. No data discrepancies were found that influenced the final interpretations.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of rotenone for male F344/N rats, as indicated by an increased incidence of parathyroid gland adenomas (uncommon tumors). There was *no evidence of carcinogenic activity* in female F344/N rats fed diets containing 38 or 75 ppm rotenone. There was *no evidence of carcinogenic activity* for male or female B6C3F$_1$ mice fed diets containing 600 or 1,200 ppm rotenone for 2 years. The decreased incidence of liver neoplasms in male mice may have been related to the administration of rotenone.

Synonym: 1,2,12,12a-tetrahydro-8,9-dimethoxy-2-1-methylethenyl)-[1]benzopyrano[3,4-b]furo[2,3-h][1]benzopyran-6(6H)-one

Trade Names of Formulations: Derrin; Derris; Tubatoxin; Nicouline; Prentox; Noxfish; Rotocide; Barbasco; Cube Root; Haiari; Dactinol

Report Date: January 1988

TR-321 Toxicology and Carcinogenesis Studies of Bromodichloromethane (CAS No. 75-27-4) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Bromodichloromethane (99% pure), one of several trihalomethanes commonly formed after chlorination of water, was selected for study because no carcinogenicity data were available for this compound and because chloroform, a related trihalomethane, had been found to cause tumors in rodents. The general population might be exposed to bromodichloromethane in drinking water supplies, in swimming pools, and in a variety of food substances.

Single-administration, 14-day, 13-week, and 2-year studies were conducted in F344/N rats and B6C3F$_1$ mice. The chemical was administered by gavage in corn oil because human exposure is primarily oral. Additional studies were performed to evaluate the potential for genetic damage in bacteria and mammalian cells.

Results of the Short-Term Studies: In the single-administration studies, the chemical was administered at doses of 150-2,500 mg/kg per day. All rats and female mice at 1,250 and 2,500 mg/kg and all male mice at 600, 1,250, and 2,500 mg/kg died; 2/5 male rats, 1/5 female rats, and 2/5 female mice at 600 mg/kg died; all animals at lower dose levels survived.

In the 14-day studies, rats received doses of 38-600 mg/kg, and mice received doses of 19-300 mg/kg per day. One female rat at 38 mg/kg and one female rat at 600 mg/kg died. Weight loss or decreased weight gain was seen at 300 and 600 mg/kg in male and female rats. All male mice at 150 and 300 mg/kg died, and one female mouse at 300 mg/kg died; no weight effects were observed in surviving mice. Dose-related necropsy findings included reddened renal medullae in male rats at 600 mg/kg and in male mice at 150 and 300 mg/kg. Clinical signs seen in high dose groups after dosing were hyperactivity in rats and lethargy in mice.

In the 13-week studies, male and female rats received doses of 19-300 mg/kg per day, male mice received doses

of 6.25-100 mg/kg per day, and female mice received doses of 25-400 mg/kg per day. Five of 10 male rats and 2/10 female rats at 300 mg/kg died. None of the mice died. Final body weights of male and female rats at 150 and 300 mg/kg were lower than those of vehicle controls (45%-88% of vehicle control weights); final body weights of male mice at 100 mg/kg and female mice at 400 mg/kg were 92% and 94% of those of the vehicle controls. Centrilobular degeneration in the liver and degeneration and necrosis of the kidney were seen in male rats at 300 mg/kg; centrilobular degeneration was seen in female rats at 300 mg/kg; degeneration and necrosis of the kidney were seen in male mice at 100 mg/kg, and centrilobular degeneration of the liver was seen in female mice at 200 and 400 mg/kg.

Experimental Design of the Two-Year Studies: The 2-year toxicology and carcinogenesis studies of bromodichloromethane were conducted by administering the chemical in corn oil by gavage, 5 days per week for 102 weeks, to groups of 50 male and female rats at doses of 0, 50, or 100 mg/kg per day; to groups of 50 male mice at doses of 0, 25, or 50 mg/kg per day; and to groups of 50 female mice at doses of 0, 75, or 150 mg/kg per day. The study in male rats was restarted because at 10.5 months into the original study, a temperature elevation killed 45/50 vehicle control male rats.

Survival and Body Weight in the Two-Year Studies: Final survival of dosed rats was comparable to that of vehicle controls (male: vehicle control, 28/50; low dose, 36/50; high dose, 28/50; female: 34/50; 27/50; 41/50). Mean body weights of high dose male and female rats were decreased during the last 1.5 years of the study; final mean body weights of high dose male and female rats were 88% and 79% of the vehicle control mean weights. Final mean body weights of low dose male and female rats were comparable to those of the vehicle controls.

Final survival of dosed male mice was comparable to that of the vehicle controls (34/50; 32/50; 42/50). At week 84, survival of female mice was greater than 50% in all dose groups. After week 84, survival of dosed and vehicle control female mice was reduced (final survival: 26/50; 13/50; 15/50), and this decreased survival was associated with ovarian abscesses (8/50; 19/47; 18/49). The final mean body weight of high dose male mice was 95% that of the vehicle controls; the final mean body weight of low dose male mice was comparable to that of the vehicle controls. Mean body weights of the high dose female mice were decreased during the last 1.5 years of the study; the final mean body weight was 75% that of the vehicle controls. The final mean body weight of the low dose female mice was 91% that of the vehicle controls.

Nonneoplastic Effects in the Two-Year Studies: Compound-related nonneoplastic lesions included cytomegaly and tubular cell hyperplasia of the kidney and necrosis and fatty metamorphosis of the liver in male rats; eosinophilic cytoplasmic change, clear cell change, focal cellular change, and fatty metamorphosis of the liver and tubular cell hyperplasia of the kidney in female rats; fatty metamorphosis of the liver, renal cytomegaly, and follicular cell hyperplasia of the thyroid gland in male mice; and follicular cell hyperplasia of the thyroid gland in female mice.

Neoplastic Effects in the Two-Year Studies: Bromodichloromethane caused compound-related increases in the incidences of neoplasms of the large intestine and kidney in male and female rats, the kidney in male mice, and the liver in female mice, as shown in the table (see page 5 of the Technical Report). The neoplasms of the large intestine and kidney are uncommon tumors in F344/N rats and $B6C3F_1$ mice.

Administration of bromodichloromethane was also associated with a decrease in the tumors of the adrenal glands in male rats, the pituitary and mammary glands in female rats, and the pituitary gland in female mice.

Genetic Toxicology: Bromodichloromethane was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested by the preincubational protocol at concentrations up to 1,000 μg/plate with or without metabolic activation. The compound was not mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay in the presence of S9 but did induce forward mutations in the system in the presence of metabolic activation from rat liver S9. Cytogenetic tests with Chinese hamster ovary cells demonstrated no induction of chromosomal aberrations or sister chromatid exchanges following treatment with bromodichloromethane in either the presence or absence of metabolic activation.

Data Audit: An audit of the experimental data was conducted for the 2-year toxicology and carcinogenesis studies of bromodichloromethane. No discrepancies were found that influenced the final interpretations of the results of these studies.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* for male and female F344/N rats and $B6C3F_1$ mice as shown by increased incidences of tubular cell adenomas and adenocarcinomas in the kidney and adenocarcinomas and adenomatous polyps in the large intestine in male and female rats, increased incidences of tubular cell adenomas and adenocarcinomas in the kidney of male mice, and increased incidences of hepatocellular adenomas and carcinomas in female mice.

Synonym: dichlorobromoethane

Report Date: October 1987

TR-322 Toxicology and Carcinogenesis Studies of Phenylephrine Hydrochloride (CAS No. 61-76-7) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Phenylephrine hydrochloride is a sympathomimetic amine recommended for use as a nasal decongestant and as a mydriatic in ophthalmic applications. In 1977, total U.S. human exposure was estimated at 1.9 x 107 g per year. Phenylephrine hydrochloride was nominated for toxicology and carcinogenesis studies because of a lack of previous long-term studies and because two other sym-

pathomimetic agents (soterenol hydrochloride and mesuprine hydrochloride) produced mesovarial leiomyomas in Sprague-Dawley rats.

Toxicology and carcinogenesis studies of USP-grade phenylephrine hydrochloride were conducted by administering diets containing the chemical (99% pure) to F344/N rats and $B6C3F_1$ mice of each sex in studies of 14 days, 12 weeks, and 2 years. In the 14-day studies, no toxic effects were seen in rats or mice fed diets containing up to 2,000 ppm phenylephrine hydrochloride. Doses were increased in the 12-week studies, and deaths of male rats and male mice were observed in groups fed diets containing 10,000 or 20,000 ppm; 1/10 male rats in the 5,000-ppm group died. Other than inflammatory eye lesions (considered secondary to the pharmacologic drying action of the chemical), no specific organ toxicity was noted. Body weights decreased as concentrations of phenylephrine hydrochloride in the diet were increased, and feed consumption was lower in dosed rats. Doses of 0, 620, and 1,250 ppm for rats and 0, 1,250, and 2,500 ppm for mice were selected for the 2-year studies because of the decreased body weight gains in animals given higher doses in the 12-week studies. In the 2-year studies, the approximate amount of phenylephrine hydrochloride consumed per day was 24 mg/kg for low dose rats, 50 mg/kg for high dose rats, 133 mg/kg for low dose mice, and 270 mg/kg for high dose mice.

Body weight differences in rats appeared to be dose related, and dosed animals were 3%-15% lighter than controls. Body weights of dosed mice averaged 3%-14% lower than those of controls throughout the 2-year studies. Survival of high dose male rats was greater than that of the controls (control, 30/50; low dose, 33/50; high dose, 42/50); differences in survival were not significant for female rats (42/50; 34/50; 36/50), male mice (35/50; 38/50; 43/50), or female mice (37/50; 34/50; 34/50).

Few nonneoplastic lesions were related to phenylephrine hydrochloride dosing in rats or mice. Chronic focal inflammation of the liver was observed at increased incidences in dosed rats (male: 2/50; 13/50; 17/50; female: 17/50; 28/50; 35/50). Inflammation of the prostate was seen more frequently in dosed than in control males (10/50; 24/50; 24/50). The incidence of focal cellular change in the liver was increased slightly in high dose male mice (0/50; 2/50; 7/50).

In male rats, mononuclear cell leukemia (24/50; 9/50; 5/50) and pheochromocytomas of the adrenal gland (14/49; 11/50; 2/50) occurred with negative trends, and the incidences in the high dose group were lower than those in the controls. No increases in neoplasia were seen in dosed male or female rats or mice.

Phenylephrine hydrochloride was not mutagenic in four strains of *Salmonella typhimurium* (TA100, TA1535, TA1537, and TA98) with or without Aroclor 1254-induced liver S9 from male Sprague-Dawley rats or male Syrian hamsters. The results of mutagenicity studies of phenylephrine hydrochloride were equivocal in the mouse lymphoma L5178Y/$TK^{+/-}$ assay in the absence of S9; it was not tested in the presence of S9. Phenylephrine hydrochloride induced sister-chromatid exchanges (SCEs) but not chromosomal aberrations in Chinese hamster ovary cells. The increase in SCEs was seen only in the absence of metabolic activation with S9.

An audit of the experimental data was conducted for the 2-year studies of phenylephrine hydrochloride. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year studies, there was *no evidence of carcinogenicity* of phenylephrine hydrochloride for male $B6C3F_1$ mice given 1,250 or 2,500 ppm in feed. Survival of high dose male rats was greater than that of controls, and the incidences of mononuclear cell leukemia and pheochromocytomas were lower in dosed than in control male rats. Inflammation was observed more frequently in the liver and prostate gland of dosed male rats than in controls.

Synonyms: benzene methanol,3-hydroxy-α[(methylamino)methyl]hydrochloride ®-; (-)-*meta*-hydroxy-α-[(methylamino)methyl]benzyl alcohol hydrochloride; *meta*-Synephrine hydrochloride; Neo-synephrine®

Report Date: January 1987

TR-323 Toxicology and Carcinogenesis Studies of Dimethyl Methylphosphonate (CAS No. 756-79-6) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Dimethyl methylphosphonate (98% pure) is one of four chemicals nominated by the U.S. Army for toxicology and carcinogenesis studies because it was being considered for use to simulate the physical and spectroscopic (but not the biologic) properties of anticholinesterase (nerve) agents. Dimethyl methylphosphonate is also used as a flame retardant, a preignition additive for gasoline, an antifoam agent, a plasticizer and stabilizer, a textile conditioner and antistatic agent, and an additive for solvents and low-temperature hydraulic fluids. The United States produces 0.2-2 million pounds (91,000-910,000 kg) of per year. Gavage was chosen as the route of administration for all four candidate "simulants" to mimic potential exposure.

Experimental Design: Dimethyl methylphosphonate was administered in corn oil by gavage to male and female F344/N rats and $B6C3F_1$ mice in single-administration, 15-day, and 13-week studies to obtain toxicity data, to establish dose levels for the 2-year studies, and to identify target tissues. Additional studies were also performed to determine toxicity to the reproductive system of male F344/N rats and $B6C3F_1$ mice and to study the potential for genetic damage in bacteria, mammalian cells, and Drosophila.

Single-Administration Studies: In the single-administration studies, dimethyl methylphosphonate was given to rats and mice at doses up to 6,810 mg/kg body weight. No compound-related deaths were seen in male or female rats or male mice; two high dose female mice died. Rats exhibited inactivity, unsteady gait, and prostration after dosing; mice were inactive after dosing.

Fifteen-Day Studies: Rats and mice received doses of 0, 1,250, 2,500, 5,000, 10,000, or 15,000 mg/kg dimethyl methylphosphonate per day. Compound-related deaths occurred in the three highest dose groups of rats and the two highest dose groups of mice. Rats receiving doses of 2,500 mg/kg or higher were inactive and at 5,000 or 10,000 mg/kg had an unsteady gait after dosing; mice exhibited inactivity, shallow breathing, and prostration at doses of 10,000 mg/kg or higher. No lesions were reported in rats. Nonneoplastic lesions of the stomach were seen in some male mice at doses of 1,250 mg/kg and higher and in some female mice at doses of 5,000 mg/kg and higher.

Thirteen-Week Studies: Dimethyl methylphosphonate was given at doses up to 8,000 mg/kg per day. Compound-related deaths occurred at 2,000, 4,000, and 8,000 mg/kg in rats and at 4,000 and 8,000 mg/kg in mice. Mean body weights of rats at 1,000 mg/kg and mice at 2,000 mg/kg were similar to those of the vehicle controls; decreased weight gain was seen at higher doses. No compound-related clinical signs were reported. Minimal to mild renal and testicular lesions were seen at all doses in male rats, but the severity of these lesions did not increase with increasing dose of the chemical. No apparent target tissues were identified in female rats or male and female mice.

Doses selected for the 2-year studies were based on body weight effects and mortality seen in the 13-week studies; the lesions seen in the kidney of male rats at the end of the 13-week studies were judged not to be life threatening. In the 2-year studies, dimethyl methylphosphonate was administered in corn oil by gavage at doses of 0, 500, or 1,000 mg/kg per day to groups of 50 F344/N rats of each sex and at 0, 1,000, or 2,000 mg/kg per day to groups of 50 $B6C3F_1$ mice of each sex. All animals were dosed 5 days per week for 103 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male rats were 5%-10% lower than those of the vehicle controls between weeks 28 and 76 and were 10%-24% lower between weeks 80 and 104. Mean body weights of high dose female rats were 8%-12% lower than those of the vehicle controls after week 80. Survival of male rats was greater than 50% in all groups until week 80, and after this time, survival decreased in both groups, with the survival at the end of the study being 27/50 in vehicle control, 17/50 in low dose, and 4/50 in high dose groups. Survival of low dose female rats was comparable to that of the vehicle controls, but the final survival of high dose female rats was decreased (vehicle control, 30/50; low dose, 33/50; high dose, 23/50). No other compound-related clinical signs were observed.

Mean body weights of high dose male mice were 7%-16% lower than those of the vehicle control males between weeks 36 and 76, and those of high dose female mice were 6%-12% lower between weeks 88 and 103. Decreased survival between weeks 23 and 45 in high dose male mice was associated with fighting. Seventeen high dose male and 22 high dose female mice died during week 45; these deaths were associated with the accidental administration of a dose mixture that had a concentration 34% greater than the targeted amount. Eleven low dose male mice died on the same day during week 77. By the end of the study, 29/50 vehicle control, 12/50 low dose, and 0/50 high dose male mice were alive; 41/50, 30/50, and 2/50 female mice survived to the end of the study.

Renal Effects in the Two-Year Studies: Administration of dimethyl methylphosphonate to male rats increased the average severity of nephropathy and caused mineralization (calcification) of the collecting tubules in the renal papilla (12/50; 41/50; 36/49), hyperplasia of the transitional epithelium lining the renal pelvis and overlying the renal papilla (0/50; 23/50; 21/49), and focal hyperplasia of the renal tubular epithelium (0/50; 8/50; 9/49). Administration of dimethyl methylphosphonate to male rats was also associated with the occurrence of rare renal tubular cell adenocarcinomas (0/50; 2/50; 3/49) and papillomas of the transitional epithelium lining of the renal pelvis (0/50; 2/50; 3/49); a transitional cell carcinoma occurred in a low dose male rat. There were no tubular cell or transitional cell neoplasms of the kidney in female rats.

Hematopoietic System Effects in the Two-Year Studies: The incidence of mononuclear cell leukemia was increased in high dose male rats (10/50; 11/50; 17/50).

Genetic Toxicity: Dimethyl methylphosphonate was not mutagenic when tested in the *Salmonella typhimurium*/microsome assay by the preincubational protocol with strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation. The chemical did induce forward mutations in the mouse lymphoma L5178Y/$TK^{+/-}$ assay system in the absence of metabolic activation. Treatment of cultured Chinese hamster ovary cells with dimethyl methylphosphonate did not induce chromosomal aberrations; however, sister chromatid exchanges were induced after exposure to this chemical in both the presence and absence of metabolic activation. When fed to Drosophila, dimethyl methylphosphonate induced a significant increase in the frequency of sex-linked recessive lethal mutations but did not induce reciprocal translocations. Dimethyl methylphosphonate caused a dominant lethal effect in male rats and mice.

Studies of Reproductive Effects: Dimethyl methylphosphonate caused a dose-related increase in the number of fetal resorptions in undosed female rats and mice mated with males that received the chemical by gavage in water 5 days per week for 13 weeks at doses of 0-2,000 mg/kg per day. After the 13-week dosing period, histopathologic changes were seen in the kidney and testis of male rats but not in male mice; dosed male rats sired fewer litters and fewer pups per litter. Dose-related decreases in sperm count and sperm motility occurred in male rats but not in male mice. Toxic effects to the reproductive system of male rats and mice were reversible after a 13- to 14-week recovery period.

Data Audit: An audit of the experimental data was conducted for the 2-year studies on dimethyl methylphosphonate. No data discrepancies were found that influenced the final interpretations.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of car-*

cinogenic activity of dimethyl methylphosphonate for male F344/N rats as shown by increased incidences of tubular cell hyperplasia, tubular cell adenocarcinomas, hyperplasia of the transitional cell epithelium, and transitional cell papillomas of the kidney. There was an increased incidence of mononuclear cell leukemia in male rats at 1,000 mg/kg. Renal toxicity and decreased survival occurred in dosed male rats. There was *no evidence of carcinogenic activity* of dimethyl methylphosphonate for female F344/N rats given doses of 500 or 1,000 mg/kg. The study in male $B6C3F_1$ mice was an *inadequate study of carcinogenic activity* because of decreased survival in both dosed groups. There was *no evidence of carcinogenic activity* for female $B6C3F_1$ mice receiving dimethyl methylphosphonate at 1,000 mg/kg; decreased survival of female mice at 2,000 mg/kg made this group inadequate for determination of carcinogenic activity.

Synonyms: fyrol DMMP; methyl phosphonic acid, dimethyl ester; DMMP; methanephosphonic acid dimethyl ester; dimethyl methanephosphonate

Report Date: November 1987

TR-324 Toxicology and Carcinogenesis Studies of Boric Acid (CAS No. 10043-35-3) in $B6C3F_1$ Mice (Feed Studies)

Boric acid is a component of cosmetics and pharmaceuticals and is also used in numerous industrial processes. Earlier long-term studies did not demonstrate a carcinogenic effect in Sprague-Dawley rats. Because of potential widespread human exposure, corroborative evidence was sought in a second species. Toxicology and carcinogenesis studies were conducted by feeding technical-grade boric acid (99.7% pure) to groups of male and female $B6C3F_1$ mice for 14 days, 13 weeks, and 2 years.

In the 14-day studies (five mice per group), mortality occurred in mice fed 25,000 ppm, 50,000 ppm, or 100,000 ppm boric acid; hyperplasia and/or dysplasia of the forestomach was also seen in these dose groups. No compound-related gross pathologic or histopathologic effects were seen in male or female mice exposed at concentrations up to 12,500 ppm in feed. In the 13-week studies, groups of 10 male and 10 female mice were fed boric acid at concentrations up to 20,000 ppm; 8 male mice and 1 female mouse receiving 20,000 ppm and 1 male receiving 10,000 ppm boric acid died before the end of the studies. Male and female mice receiving 20,000 ppm boric acid weighed 23% and 18% less, respectively, than did the controls at the end of the studies. Testicular atrophy in 8/10 male mice, hyperkeratosis and acanthosis of the stomach in 8/10 male and female mice, and extramedullary hematopoiesis of the spleen in all male and female mice receiving 20,000 ppm boric acid indicated that the testis, stomach, and spleen were potential target organs in the 2-year studies. Based on these results, 2-year toxicology and carcinogenesis studies were conducted by feeding diets containing boric acid at concentrations of 0, 2,500, or 5,000 ppm to groups of 50 male and 50 female mice.

Survival of high dose male mice after week 63 and of low dose mice after week 84 was lower than that of the controls (final survival: control, 41; low dose, 30; high dose, 22), which may have reduced the sensitivity of the carcinogenicity study; the numbers of female mice (33; 33; 37) that survived to the end of the studies were considered adequate for toxicologic evaluation. Body weight gain was reduced in each sex after week 30; mean final body weights were 7% and 13% below control values for exposed male mice and 7% and 20% below those of controls for exposed female mice. No chemically related clinical signs were reported.

At the top dose, boric acid caused an increased incidence of testicular atrophy (control, 3/49; low dose, 6/50; high dose, 27/47) and interstitial cell hyperplasia (0/49; 0/50; 7/47) in male mice. The testicular atrophy was characterized by variable loss of spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa from the seminiferous tubules. The seminiferous tubules contained primarily Sertoli cells and variable numbers of spermatogonia. In some mice, there were accumulations of interstitial cells, indicating hyperplasia.

In low dose male mice, there were increased incidences of hepatocellular carcinomas (5/50; 12/50; 8/49) and hepatocellular adenomas or carcinomas (combined) (14/50; 19/50; 15/49) and an increased incidence of subcutaneous tissue fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) (2/50; 10/50; 2/50). No increased incidence of subcutaneous tissue neoplasms was seen in male mice receiving 5,000 ppm. Because the incidence of subcutaneous tissue tumors is variable in historical controls, because there was no corresponding increase in the high dose male mice, and because the incidence of hepatocellular tumors was not significant by the incidental tumor test and was within the historical control range, neither of these tumors was considered to be related to the administration of boric acid.

Boric acid was not mutagenic in the Salmonella/microsome assay with *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537. Boric acid was negative in the mouse lymphoma L5178Y/$TK^{+/-}$ assay and did not induce sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells. All assays were preformed with and without metabolic activation.

The data, documents, and pathology materials from the 2-year studies of boric acid were audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenicity* of boric acid at doses of 2,500 or 5,000 ppm for male or female $B6C3F_1$ mice. Testicular atrophy and interstitial cell hyperplasia

were observed in high dose male mice. The decrease in survival of dosed male mice may have reduced the sensitivity of this study.

Synonyms: orthoboric acid; boracic acid

Report Date: October 1987

TR-325 Toxicology and Carcinogenesis Studies of Pentachloronitrobenzene (CAS No. 82-68-8) in B6C3F$_1$ Mice (Feed Studies)

Toxicology and carcinogenesis studies of pentachloronitrobenzene (99% pure), a fungicide, were conducted by administering diets containing 0, 2,500, or 5,000 ppm pentachloronitrobenzene to groups of 50 B6C3F$_1$ mice of each sex for 103 weeks. These doses were selected because, in 13-week studies in which the chemical was administered in feed at doses up to 20,000 ppm in male mice and up to 40,000 ppm in female mice, body weight gain depression was observed at 10,000 ppm and above in males and female and deaths occurred at 40,000 ppm in females.

The National Cancer Institute had conducted 2-year (diet) studies in B6C3F$_1$ mice and Osborne-Mendel rats (See TR-61 reported in 1978). Survival among male mice was low, not all livers were examined from dosed female mice, and the size of the control group was considered to be small. For these reasons, the NCI decided to conduct additional 13-week and 2-year studies in B6C3F$_1$ mice. Under the conditions of the NCI studies, pentachloronitrobenzene was not carcinogenic in either Osborne-Mendel rats or B6C3F$_1$ mice.

In the studies reported in this Technical Report, the survival of male mice was comparable among control and dosed groups (control, 35/50; low dose, 31/50; high dose, 32/50). Final mean body weights of low dose and high dose male mice were 96% and 90% that of the controls. All groups of female mice showed evidence of bacterial infection. At week 84, survival in dosed and control female mice was 38/50; 34/50; 30/50; after week 84, survival in dosed groups decreased, with the final survival being 30/50; 20/50; 15/50. The mean body weight of high dose female mice was more than 10% lower than that of the control group after week 20 and was 21% lower than controls at week 104. The mean body weight of low dose female mice was within 10% that of the control group until week 88 and was 18% lower than controls at week 104.

No compound-related neoplastic lesions were seen in either male or female mice. The nonneoplastic lesions observed in female mice were considered to be secondary to bacterial infection (primarily Klebsiella) and included hematopoiesis of the liver (9/50; 21/50; 23/50) and spleen (14/50; 23/48; 27/50), plasma cell hyperplasia of the mediastinal lymph nodes (1/44; 4/47; 9/45), and ovarian abscesses (12/49; 22/50; 29/50).

Pentachloronitrobenzene was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of Aroclor 1254-induced male Syrian hamster or male Sprague-Dawley rat liver S9 when tested according to the preincubational protocol. Pentachloronitrobenzene was not mutagenic at the TK$^{+/-}$ locus of L5178Y mouse lymphoma cells in the presence or absence of Aroclor 1254-induced F344/N rat liver S9. In cultured Chinese hamster ovary cells, pentachloronitrobenzene did not induce sister-chromatid exchanges but did induce chromosomal aberrations both with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9.

An audit of the experimental data was conducted for the 2-year studies of pentachloronitrobenzene. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenicity* for either male or female B6C3F$_1$ mice receiving 2,500 or 5,000 ppm of pentachloronitrobenzene. Infection is considered to have decreased survival of the female mice and thus reduced the sensitivity for determining the presence or absence of a carcinogenic response.

Synonyms or Trade Names: Avicol®; PCNB; quintozene; Botrilex®; Brassicol®;folosan®; PKhNB; Tilcarex®; Terraclor®; Tritosan®

Report Date: January 1987

TR-326 Toxicology and Carcinogenesis Studies of Ethylene Oxide (CAS No. 75-21-8) in B6C3F$_1$ Mice (Inhalation Studies)

Ethylene oxide is a major industrial chemical used primarily as an intermediate in the manufacture of other chemicals; e.g., ethylene glycol, a major component of automotive and other antifreeze products. Exposure to ethylene oxide is greatest in the health care industry, where an estimated 75,000 workers are potentially exposed. Ethylene oxide was nominated for toxicology and carcinogenesis studies in B6C3F$_1$ mice because of its extensive production; the potential for human exposure in the workplace, from medical devices, or from food; the positive results of genetic toxicology assays; and the previous use of only F344/N rats in inhalation carcinogenicity studies.

Two inhalation studies reported in 1984 by Snellings et al. and by Lynch et al. demonstrated carcinogenic responses in F344/N rats. Results were similar in both studies and consisted of increased incidences of mononuclear cell leukemia, peritoneal mesotheliomas, and primary brain tumors.

Experimental Design: Toxicology and carcinogenesis studies of ethylene oxide (greater than 99% pure) were conducted by exposing groups of 50 B6C3F$_1$ mice of each sex to air containing 0, 50, or 100 ppm ethylene oxide, 6 hours per day, 5 days per week for 102 weeks. These doses were selected because, in 14-week studies, all mice

exposed at 600 ppm died within 1 week, and all mice exposed at 400 ppm died by week 4. Rhinitis was observed in both sexes exposed at 200, 400, and 600 ppm as was renal tubular degeneration in both sexes at 100, 200, and 400 ppm. The latter effects observed at 100 ppm were slight and deemed not to be life threatening in 2-year studies.

Two-Year Studies: Survival of exposed and control mice was comparable in the 2-year studies (male: control, 28/50; low dose, 31/50; high dose, 34/50; female: 25/50; 24/50; 31/50). Final mean body weights in exposed mice were 95%-102% of those of the controls. No compound-related clinical signs were observed.

Those neoplastic lesions that occurred at elevated incidences in mice exposed to ethylene oxide are reported in the following table (see page 6 of the Technical Report). In male mice, alveolar/bronchiolar carcinomas, alveolar/bronchiolar adenomas, and papillary cystadenomas of the harderian gland occurred with positive trends. In female mice, alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, papillary cystadenomas of the harderian gland, malignant lymphomas, and uterine adenocarcinomas occurred with positive trends. Mammary gland tumors also were increased in exposed female mice.

Data Audit: An audit of the experimental data was conducted for the 2-year studies of ethylene oxide. No data discrepancies were found that influenced the final interpretations.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity* for $B6C3F_1$ mice as indicated by dose-related increased incidences of benign or malignant neoplasms of the lung and benign neoplasms of the harderian gland in both male and female $B6C3F_1$ mice following exposure to ethylene oxide vapors at 50 and 100 ppm. In female mice, ethylene oxide caused additional malignant neoplasms of the uterus, mammary gland, and hematopoietic system (lymphoma).

Synonyms: oxirane; EO; ETO; dihydrooxirene; dimethylene oxide; 1,2-epoxyethane; oxane; α,β-oxidoethane

Report Date: November 1987

TR-327 Toxicology and Carcinogenesis Studies of Xylenes (Mixed) (60% *m*-Xylene, 14% *p*-Xylene, 9% *o*-Xylene, and 17% Ethylbenzene) (CAS No. 1330-20-7) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

The technical grade of xylenes (mixed) (hereafter termed xylenes) contains the three isomeric forms and ethylbenzene (percentage composition shown above). The annual production for 1985 was approximately 7.4 x 10^8 gallons. Xylenes is used as a solvent and a cleaning agent and as a degreaser and is a constituent of aviation and automobile fuels. Xylenes is also used in the production of benzoic acid, phthalate anhydride, and isophthalic and terephthalic acids as well as their dimethyl esters.

Toxicology and carcinogenesis studies of xylenes were conducted in laboratory animals because a large number of workers are exposed and because the long-term effects of exposure to xylenes were not known. Exposure for the present studies was by gavage in corn oil. In single-administration studies, groups of five F344/N rats and $B6C3F_1$ mice of each sex received 500, 1,000, 2,000, 4,000, or 6,000 mg/kg. Administration of xylenes caused deaths at 6,000 mg/kg in rats and mice of each sex and at 4,000 mg/kg in male rats. In rats, clinical signs observed within 24 hours of dosing at 4,000 mg/kg included prostration, muscular incoordination, and loss of hind limb movement; these effects continued through the second week of observation. Tremors, prone position, and slowed breathing were recorded for mice on day 3, but all mice appeared normal by the end of the 2-week observation period. In 14-day studies, groups of five rats of each sex were administered 0, 125, 250, 500, 1,000, or 2,000 mg/kg, and groups of five mice of each sex received 0, 250, 500, 1,000, 2,000, or 4,000 mg/kg. Chemical-related mortality occurred only at 2,000 mg/kg in rats and at 4,000 mg/kg in mice. Rats and mice exhibited shallow breathing and prostration within 48 hours following dosing at 2,000 mg/kg. These signs persisted until day 12 for rats, but no clinical signs were noted during the second week for mice. In 13-week studies, groups of 10 rats of each sex received 0, 62.5, 125, 250, 500, or 1,000 mg/kg, and groups of 10 mice of each sex received 0, 125, 250, 500, 1,000, or 2,000 mg/kg. No deaths or clinical signs of toxicity were recorded in rats. However, high dose male rats gained 15% less weight and females 8% less weight than did the vehicle controls. Two female mice died at the 2,000 mg/kg dose. Lethargy, short and shallow breathing, unsteadiness, tremors, and paresis were observed for both sexes in the 2,000 mg/kg group within 5-10 minutes after dosing and lasted for 15-60 minutes.

Two-year toxicology and carcinogenesis studies were conducted by administering 0, 250, or 500 mg/kg xylenes in corn oil by gavage to groups of 50 F344/N rats of each sex, 5 days per week for 103 weeks. Groups of 50 $B6C3F_1$ mice of each sex were administered 0, 500, or 1,000 mg/kg xylenes on the same schedule. Although the mortality was dose related in male rats (final survival: vehicle control, 36/50; low dose, 26/50; high dose, 20/50), many of the early deaths in the dosed males were gavage related. Body weights of the high dose male rats were 5%-8% lower than those of the vehicle controls after week 59. The mean body weights of low dose and vehicle control male rats and those of dosed and vehicle control female rats were comparable. Survival rates of female rats and both sexes of dosed mice were not significantly different from those of the vehicle controls. The mean weights of dosed male and female mice were comparable to those of the vehicle controls. Hyperactivity lasting 5-30 minutes was observed in high dose mice after dosing, beginning after week 4 and continuing through week 103.

At no site was the incidence of nonneoplastic or neoplastic lesions in dosed rats or mice of either sex considered to be related to the administration of xylenes.

Neither xylenes nor any of its components (*o*-xylene, *m*-xylene, *p*-xylene, or ethylbenzene) were mutagenic when tested with or without metabolic activation in *Salmonella typhimurium* strains TA100, TA1535, TA97, or TA98 with the preincubation protocol. In addition, ethylbenzene was tested in cytogenetic assays using cultured Chinese hamster ovary cells both with and without metabolic activation; neither sister-chromatid exchanges nor chromosomal aberrations were induced by ethylbenzene.

An audit of the experimental data was conducted for the 2-year studies of xylenes. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenicity* of xylenes (mixed) for male or female F344/N rats given 250 or 500 mg/kg or for male or female $B6C3F_1$ mice given 500 or 1,000 mg/kg.

Report Date: December 1986

TR-328 Toxicology and Carcinogenesis Studies of Methyl Carbamate (CAS No. 598-55-0) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Methyl carbamate is used as a chemical intermediate by the textile industry for the manufacture of dimethylol methyl carbamate-based resins that are applied on polyester/cotton blend fabrics as durable-press finishes.

Experimental Design: Toxicology and carcinogenesis studies of methyl carbamate (98% pure) were conducted by exposing groups of F344/N rats and $B6C3F_1$ mice by gavage in water in a single dose and by repeated administration for 16 days, 13 weeks, 6 months, 12 months, 18 months, and 2 years. In addition, short-term mutagenicity studies in bacteria, mammalian cells, and Drosophila and of unscheduled DNA synthesis in rat liver cells were conducted.

Single-Administration Studies: In the single-administration studies, 5/5 male and 5/5 female rats that received 8,000 mg/kg methyl carbamate and 2/5 male and 5/5 female that received 4,000 mg/kg died before the end of the 15-day observational period. Five of five male and 5/5 female mice that received 8,000 mg/kg and 1/5 females that received 4,000 mg/kg died before the end of the 15-day observational period. No compound-related morphologic effects were observed in rats or mice that received 2,000 mg/kg.

Sixteen-Day Studies: In the 16-day studies, all rats dosed at 2,000 or 4,000 mg/kg died, and 3/5 male rats that received 1,000 mg/kg died. Male mice that received 2,000 or 4,000 mg/kg, female mice that received 4,000 mg/kg, and 1/5 female mice that received 2,000 mg/kg died. No compound-related gross pathologic or histopathologic effects were seen in male or female rats (groups of five each) that received 500 mg/kg or in mice that received 1,000 mg/kg.

Thirteen-Week Studies: In the 13-week studies, groups of 10 male and 10 female rats and mice received up to 800 mg/kg (male rats), 1,000 mg/kg (female rats), 1,500 mg/kg (male mice), or 2,000 mg/kg (female mice). Four of 10 male rats that received 800 mg/kg and 1/10 female rats that received 1,000 mg/kg died of compound-related causes before the end of the studies. Toxic hepatitis, splenic pigmentation, bone marrow atrophy, and testicular atrophy were observed in the two highest dose groups of rats. One of the female mice that received 2,000 mg/kg died. The dosed female mice had significantly greater liver weights than did the vehicle controls.

Experimental Design of Six-, Twelve-, and Eighteen-Month and Two-Year Studies: Based on the findings in the short- term studies, 2-year studies of methyl carbamate were conducted by administering 0, 100, or 200 mg/kg methyl carbamate in distilled water by gavage, 5 days per week for 103 weeks, to groups of 50 F344/N rats of each sex for 103 weeks. Groups of 50 $B6C3F_1$ mice of each sex were administered 0, 500, or 1,000 mg/kg methyl carbamate on the same schedule. Additional groups of 30 rats of each sex were administered 0 or 400 mg/kg methyl carbamate, and additional groups of 30 mice of each sex were administered 0 or 1,000 mg/kg methyl carbamate in distilled water by gavage, 5 days per week. Ten animals from each group were killed at 6, 12, or 18 months so that the progression of lesions could be followed.

Results of the Six-, Twelve-, and Eighteen-Month and Two-Year Studies: In the 6-month studies, all vehicle control and dosed (400 mg/kg) rats survived. Cytologic alterations and atypical proliferative changes were observed in the liver of all dosed male and female rats, and neoplastic nodules of the liver were observed in 6/10 dosed male and 5/10 dosed female rats. In the 12-month studies, all vehicle control male and female rats and dosed female rats survived. One of 10 dosed male rats died. Neoplastic nodules of the liver were observed in 7/10 dosed male and 9/10 dosed female rats, and hepatocellular carcinomas were observed in 8/10 dosed male and 6/10 dosed female rats. In the 18-month studies, 1/10 dosed male and 8/10 dosed female and all vehicle control rats survived. Hepatocellular carcinomas were observed in 9/10 dosed male and 8/10 dosed female rats. Compound-related neoplastic changes were not observed in mice in the 6-, 12-, or 18-month studies.

In the 2-year studies, mean body weights of high dose (200 mg/kg) male rats were generally 5%-9% lower than those of the vehicle controls after week 20. Mean body weights of high dose female rats were 5%-8% lower than those of the vehicle controls after week 56. Survival of dosed and vehicle control rats was similar (male: vehicle control, 19/50; low dose, 26/50; high dose, 29/50; female: 29/50; 36/50; 35/50). The mean body weights of high dose (1,000 mg/kg) male mice were about 8%-18% lower than those of the vehicle controls after week 24. The mean body weights of high dose (1,000 mg/kg) female mice were about 16% lower than those of the vehicle controls after week 16 and 30% lower after week 64. Survival of dosed and vehicle control mice was similar (male: 28/50; 35/50; 28/50; female: 38/50; 36/50; 32/50).

Chronic focal inflammation and cytologic alteration of the liver were observed at increased incidences in high dose rats of each sex. Hyperplasia of hepatocytes was observed at increased incidences in dosed male and high dose female rats. Neoplastic nodules or hepatocellular carcinomas (combined) in female rats occurred with a significant positive trend (0/50; 0/50; 6/49; $P<0.01$); the incidence of neoplastic nodules or hepatocellular carcinomas (combined) in high dose female rats was greater ($P<0.03$) than that in the vehicle controls. Incidences of liver neoplasms in dosed male rats were not significantly increased (4/50; 0/50; 7/49). Inflammation of the harderian gland was observed at increased incidences in dosed rats (male: 4/50; 11/50; 16/50; female: 7/50; 16/50; 30/50). The lesions were considered to be chemically related. In the 2-year studies in rats, significant decreases in tumor incidences included the following: leukemia (both sexes), pituitary gland (male), adrenal gland (male), and mammary gland (female).

In the 2-year mouse studies, multinucleate giant cells in the liver were observed at increased incidences in dosed male mice (14/50; 31/50; 31/49). Adenomatous hyperplasia and histiocytosis of the lung were observed at increased incidences in high dose mice (adenomatous hyperplasia—male: 13/50; 19/50; 24/49; female: 7/49; 10/50; 18/50; histiocytosis—male: 11/50; 7/50; 21/49; female: 9/49; 10/50; 21/50).

Genetic Toxicology: Methyl carbamate was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535 when tested with or without metabolic activation in a preincubation protocol at doses up to 10 mg/plate. Methyl carbamate did not induce forward mutations in the mouse L5178Y/TK$^{+/-}$ lymphoma assay with or without metabolic activation at doses up to 5 mg/ml. Unscheduled DNA synthesis was not detected in rat hepatocytes after in vitro treatment with methyl carbamate at concentrations of 1.0-1,000 μg/ml. When tested in Drosophila at doses of 25,000-50,000 ppm, methyl carbamate did not induce sex-linked recessive lethal mutations. Results of tests for induction of chromosomal aberrations and sister chromatid exchanges by methyl carbamate in cultured Chinese hamster ovary cells were also negative at doses up to 5 mg/ml.

Data Audit: An audit of the experimental data was conducted for the 6-, 12-, and 18-month and 2-year studies of methyl carbamate. No data discrepancies were found that influenced the final interpretation.

Conclusions: Under the conditions of these 6-, 12-, and 18-month and 2-year gavage studies, there was *clear evidence of carcinogenic activity* for male and female F344/N rats given methyl carbamate as indicated by increased incidences of hepatocellular neoplastic nodules and hepatocellular carcinomas. There was *no evidence of carcinogenic activity* for male and female B6C3F$_1$ mice given methyl carbamate at doses of 500 or 1,000 mg/kg. Methyl carbamate also induced inflammation of the harderian gland in male and female rats and adenomatous hyperplasia and histiocytosis of the lung in male and female mice.

Synonyms: carbamic acid, methyl ester; methylurethan; methylurethane; urethylane

Report Date: November 1897

TR-329 Toxicology and Carcinogenesis Studies of 1,2-Epoxybutane (CAS No. 106-88-7) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

1,2-Epoxybutane was selected for study because it is a short-chain epoxide that had been shown to be mutagenic and because no carcinogenicity data were available. Approximately 8 million pounds of 1,2-epoxybutane are produced annually in the United States. The chemical is used primarily as a stabilizer in chlorinated hydrocarbon solvents.

Single-Exposure, Fourteen-Day, and Thirteen-Week Studies: Single-exposure, 14-day, 13-week, and 2-year studies were conducted in F344/N rats and B6C3F$_1$ mice. The chemical was greater than 99% pure and was administered as a vapor by the inhalation route to mimic worker exposure; room air was used as the control exposure during these studies. Exposures were 6 hours per day (5 days per week), except in the single-exposure studies (4 hours). Additional studies were performed to evaluate the potential for genetic damage in bacteria and in mammalian cells. In the single-exposure studies, the chemical was administered at exposure concentrations of 400-6,550 ppm in rats and 400-2,050 ppm in mice. In the 14-day studies, rats and mice were exposed at 400-6,400 ppm, and in the 13-week studies, rats and mice were exposed at 50-800 ppm.

All rats in the single-exposure studies at 6,550 ppm died; compound-related deaths were not seen in other dosed groups. All mice at 2,050 ppm and 4/5 mice of each sex at 1,420 ppm died; compound-related mortality was not seen in other dosed groups.

In the 14-day studies, all rats at 3,200 and 6,400 ppm and 2/5 female rats at 1,600 ppm died; all mice at 1,600, 3,200, and 6,400 and 1/5 male mice at 800 ppm died. Final mean body weights of surviving rats exposed at 800 or 1,600 ppm were 12%-33% lower than those of the controls; final mean body weights of surviving mice at 800 ppm were 10%-12% lower than those of the controls. Compound-related lesions included pulmonary hemorrhage and rhinitis in rats at 1,600 ppm and nephrosis in mice at 800 and 1,600 ppm.

In the 13-week studies, no compound-related mortality was observed in rats; all mice exposed at 800 ppm died. No compound-related clinical signs were seen in rats or in surviving mice. The final mean body weight of rats exposed at 800 ppm was 23% lower than that of controls for males and 16% lower for females. Final body weights of surviving mice were unaffected by exposure. Inflammation of the nasal turbinates was seen in rats at 800 ppm but not at lower exposure concentrations. Renal tubular necrosis was seen in mice at 800 ppm but not at

lower concentrations. Inflammation of the nasal turbinates was observed in female mice at 100, 200, 400, and 800 ppm and in male mice at 200, 400, and 800 ppm. The highest exposure concentration selected for the 2-year studies in rats was 400 ppm because of body weight effects and nasal lesions observed at 800 ppm. The highest concentration selected for the 2-year studies in mice was 100 ppm because the nasal lesions seen at 200 and 400 ppm were considered to be potentially life threatening.

Two-Year Studies: The 2-year toxicology and carcinogenesis studies of 1,2-epoxybutane were conducted by exposing groups of 50 animals per species and sex to the chemical by inhalation, 6 hours per day 5 days per week. Rats were exposed at concentrations of 0, 200, or 400 ppm for 103 weeks and mice at 0, 50, or 100 ppm for 102 weeks.

Body Weight and Survival in the Two-Year Studies: The survival of all groups of dosed rats was at least 50% until week 98, but final survival was reduced in the dosed groups (final survival—male: control, 30/50; low dose, 18/50; high dose, 23/50; female: 32/50; 21/50; 22/50). Mean body weights of control and exposed male rats were similar until week 86; thereafter, mean body weights of high dose male rats were 4%-8% lower than those of controls. Mean body weights of high dose female rats were 5%-10% lower than those of controls after week 22.

Survival in male mice was comparable among groups (final survival: 41/50; 45/50; 33/50). Survival in female mice was greater than 50% in all groups at week 86 and then was reduced in high dose females toward the end of the study (final survival: 29/50; 25/50; 9/50). This decreased survival was associated with suppurative inflammation of the ovary and uterus. *Klebsiella oxytoca* was isolated from these ovarian/uterine lesions. Mean body weights of high dose male mice were 10%-14% lower than those of the controls after week 69; mean body weights of low dose male mice were 4%-8% lower than those of the controls after week 86. Mean body weights of high dose female mice were 13%-23% lower than those of the controls after week 60, and mean body weights of low dose female mice were 12%-16% lower than those of the controls after week 73.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Dosed rats had nonneoplastic lesions of the nasal cavity including inflammation, epithelial hyperplasia, squamous metaplasia, hyperostosis of the nasal turbinate bone, and atrophy of the olfactory epithelium. Seven papillary adenomas of the nasal cavity were seen in high dose male rats and two in high dose female rats. The historical incidences of nasal cavity adenomas in untreated male and untreated female F344/N rats are less than 0.1%. The incidences of alveolar/bronchiolar carcinomas (0/50; 1/50; 4/49) and adenomas or carcinomas (combined) (0/50; 2/50; 5/49) were increased in high dose male rats; no increased incidences of these tumors were observed in dosed female rats.

Dosed mice had increased incidences of nonneoplastic lesions of the nasal cavity but no significant increase in the incidence of neoplastic lesions of the nasal cavity. The nonneoplastic lesions included suppurative inflammation (empyema), epithelial hyperplasia, erosion, regeneration, and squamous metaplasia in the nasal cavity; atrophy of the olfactory sensory epithelium ; hyperplasia of the nasal gland (Bowman's glands); and inflammation and hyperplasia of the nasolacrimal duct. A single squamous cell papilloma was seen in the incisive duct of one high dose male mouse.

Genetic Toxicology: 1,2-Epoxybutane was mutagenic in *Salmonella typhimurium* strains TA100 and TA1535 when tested with a preincubational protocol with or without rat liver S9, indicating that it is a direct-acting mutagen capable of inducing base-pair substitutions in prokaryotes; it did not cause gene reversion in strains TA1537 or TA98. 1,2-Epoxybutane induced forward mutations at the TK locus of cultured mouse L5178Y lymphoma cells with and without metabolic activation. Both chromosomal aberrations and sister chromatid exchanges were induced in cultured Chinese hamster ovary cells after exposure to 1,2-epoxybutane in the presence and absence of metabolic activation. 1,2-Epoxybutane, when fed to male Drosophila, caused significant increases in the number of sex-linked recessive lethal mutations and reciprocal translocations in the germ cells.

Data Audit: An audit of the experimental data was conducted for the 2-year studies of 1,2-epoxybutane. No data discrepancies were found that influenced the final interpretations.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity* of 1,2-epoxybutane for male F344/N rats, as shown by an increased incidence of papillary adenomas of the nasal cavity, alveolar/bronchiolar carcinomas, and alveolar/bronchiolar adenomas and carcinomas (combined). There was *equivocal evidence of carcinogenic activity* for female F344/N rats, as shown by the presence of papillary adenomas of the nasal cavity. There was *no evidence of carcinogenic* activity for male or female $B6C3F_1$ mice exposed at 50 or 100 ppm. 1,2-Epoxybutane exposure was associated with adenomatous hyperplasia and inflammatory lesions of the nasal cavity in rats and inflammatory lesions of the nasal cavity in mice.

Synonyms: 1-butene oxide; 1,2-butene oxide; butylene oxide; 1,2-butylene oxide; ethyl ethylene oxide; ethyl oxirane

Report Date: March 1988

TR-330 Toxicology and Carcinogenesis Studies of 4-Hexylresorcinol (CAS No. 136-77-6) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

4-Hexylresorcinol, which is used as an anthelmintic and antiseptic, was nominated by the National Cancer

Institute for study. Toxicology and carcinogenesis studies were conducted by administering 4-hexylresorcinol (greater than 99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days, 13 weeks, or 2 years.

Sixteen-Day and Thirteen-Week Studies: In the 16-day studies, groups of five rats and five mice of each sex were administered 0, 31.3, 62.5, 125, 250, or 500 mg/kg 4-hexylresorcinol. Survival was not affected. Decreased body weights were seen in male rats that received 250 or 500 mg/kg 4-hexylresorcinol. No other effects were observed. In the 13-week studies, groups of 10 rats and 10 mice of each sex were administered 0, 62.5, 125, 250, 500, or 1,000 mg/kg of the chemical, 5 days per week. All rats and male mice and 9/10 female mice that received 1,000 mg/kg died before the end of the studies. Final mean body weights of male rats that received 250 or 500 mg/kg were 22% or 38% lower than that of the vehicle controls; final mean body weights of female rats that received 250 or 500 mg/kg were 16% or 9% lower. No compound-related gross or microscopic pathologic effects were observed in rats. No body weight effects were observed for mice. Mild to moderate nephropathy was dose related in male and female mice.

Based on these results, 2-year toxicology and carcinogenesis studies of 4-hexylresorcinol were conducted by administering 0, 62.5, or 125 mg/kg to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex, 5 days per week.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male rats were 7%-11% lower than those of the vehicle controls throughout the study. Mean body weights of low dose male and dosed female rats were similar to those of the vehicle controls. The body weights of dosed male and dosed female mice were comparable to those of vehicle controls except during the last 16 weeks of the studies, when body weights were 6%-16% lower in the dosed groups. No significant differences in survival were observed between any groups of rats or mice of either sex (male rats: vehicle control, 30/50; low dose, 29/50; high dose, 33/50; female rats: 28/50; 32/50; 30/50; male mice: 36/50; 26/50; 30/50; female mice: 35/50; 32/50; 35/50).

Nonneoplastic and Neoplastic Lesions in the Two-Year Studies: Two astrocytomas and an oligodendroglioma were observed in high dose male rats, a glioma was observed in one low dose male rat, and an oligodendroglioma was observed in one vehicle control male rat. These neoplasms were not considered to be related to 4-hexylresorcinol administration.

Focal medullary hyperplasia of the adrenal gland was observed at increased incidences in dosed male mice (5/50; 16/50; 10/49). Pheochromocytomas in male mice occurred with a marginal upward trend (1/50; 2/50; 5/49). Historically, these neoplasms are observed in about 1% of corn oil vehicle control B6C3F$_1$ male mice. The incidences of neoplasms of the harderian gland in male mice were slightly increased over those in the vehicle controls (adenomas or carcinomas, combined: 0/50; 4/50; 3/50).

Decreases were observed in the incidences of mononuclear cell leukemia in dosed male (12/49; 7/50; 1/50) and female (16/50; 3/50; 2/50) rats, hepatocellular adenomas or carcinomas (combined) in dosed male mice (21/50; 9/50; 9/50), and circulatory system tumors in male (10/50; 4/50; 2/50) and female (6/50; 2/49; 0/50) mice. These decreased incidences of tumors in rats and mice are considered to be possibly related to 4-hexylresorcinol administration.

The incidences and severity of nephropathy (male: 39/50; 43/50; 47/50; female: 7/50; 40/49; 47/50) and incidences of osteosclerosis (male: 5/50; 5/50; 15/50; female: 21/50; 25/49; 40/50) were increased in both dosed male and female mice and are considered to be related to chemical exposure.

Genetic Toxicology: 4-Hexylresorcinol was not mutagenic for *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without S9 metabolic activation. 4-Hexylresorcinol induced forward mutations at the TK locus in mouse L5178Y cells in the presence of S9; no response was observed in the absence of metabolic activation. In cytogenetic assays with cultured Chinese hamster ovary (CHO) cells, 4-hexylresorcinol caused an increase in the frequency of sister chromatid exchanges (SCEs) in the absence of metabolic activation; no induction of SCEs was observed in the presence of S9. Chromosomal aberrations were not induced in CHO cells with or without metabolic activation.

Data Audit: The data, documents, and pathology materials from the 2-year studies of 4-hexylresorcinol were audited at the NTP Archives. The audit findings show that the conduct of the studies is documented appropriately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of 4-hexylresorcinol for male or female F344/N rats given doses of 62.5 or 125 mg/kg. There was *equivocal evidence of carcinogenic activity* of 4-hexylresorcinol for male B6C3F$_1$ mice, as shown by marginally increased incidences of pheochromocytomas (and hyperplasia) of the adrenal medulla and of harderian gland neoplasms. There was *no evidence of carcinogenic activity* for female B6C3F$_1$ mice given doses of 62.5 or 125 mg/kg 4-hexylresorcinol. Decreased incidences of three tumors types were considered related to 4-hexylresorcinol administration: mononuclear cell leukemia in male and female rats, hepatocellular neoplasms in male mice, and circulatory system tumors in male and female mice.

Synonyms: 4-hexyl-1,3-benzenediol; 4-hexyl-1,3-dihydroxybenzene

Report Date: May 1988

TR-331 Toxicology and Carcinogenesis Studies of Malonaldehyde, Sodium Salt (3-Hydroxy-2-propenal, Sodium Salt) (CAS No. 24382-04-5) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Malonaldehyde occurs as a natural metabolic byproduct of prostaglandin biosynthesis and as an end product of polyunsaturated lipid peroxidation. Toxicology and carcinogenesis studies of malonaldehyde were conducted by administering the chemical as malonaldehyde, sodium salt, a stabilized form of malonaldehyde, in distilled water by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, and two years. The study material was 63%-79% malonaldehyde, sodium salt, 22%-38% water, and 1% or less other impurities. The water content was taken into account when the dose mixtures were prepared.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, groups of five rats and five mice of each sex were dosed with 250, 500, 750, 1,000, or 1,500 mg/kg malonaldehyde, sodium salt. Controls were untreated. Rats and mice that received 1,500 mg/kg malonaldehyde, sodium salt, did not survive to the end of the 14-day studies. No compound-related gross lesions were seen in the dosed animals.

In the 13-week studies, groups of 10 males and 10 females of each species were administered 0, 30, 60, 125, 250, or 500 mg/kg malonaldehyde, sodium salt. Nine of 10 male rats, 10/10 female rats, 3/10 male mice, and 1/10 female mice that received 500 mg/kg malonaldehyde, sodium salt, died before the end of the studies. Body weights were reduced by more than 15% in rats receiving 250 or 500 mg/kg and in mice receiving 500 mg/kg.

Compound-related nonneoplastic lesions were present in the stomach, testis, and kidney of rats and in the pancreas, stomach, and testis of mice. Focal and multifocal erosive lesions were observed in the gastric mucosa of the glandular stomach in the 500 mg/kg groups of male and female rats. Dilatation of the gastric glands of the stomach mucosa occurred in the 500 mg/kg male mice. Lesions of the kidney included membranous glomerular nephropathy in the 250 and 500 mg/kg male rats and the 125, 250, and 500 mg/kg female rats and mineralization in the 250 and 500 mg/kg male rats and the 60, 125, 250, and 500 mg/kg female rats. Degeneration of the testicular germinal epithelium was observed in male rats and male mice receiving 250 and 500 mg/kg. Atrophy of the exocrine pancreas was seen in the 125, 250, and 500 mg/kg male and the 250 and 500 mg/kg female mice.

Based on these results, 2-year studies of malonaldehyde, sodium salt, were conducted by exposing groups of 50 F344/N rats of each sex at doses of 0, 50, or 100 mg/kg, administered 5 days per week for 103 weeks. Doses of 0, 60, or 120 mg/kg were administered in the same schedule to groups of 50 male and 50 female B6C3F$_1$ mice.

Body Weight and Survival in the Two-Year Studies: Final mean body weights at the end of the study were reduced by 26% and 36% for high dose male and female rats compared with those for the vehicle controls. The final mean body weight of high dose male mice was 92% that of the vehicle controls. The final mean body weights of low dose male mice, low dose rats, and all groups of female mice were comparable to those of the vehicle controls.

The survival of high dose male and female rats was significantly lower than that of the vehicle controls, with survival declining rapidly after week 76 for high dose males and after week 59 for high dose females (survival—male: vehicle control, 37/50; low dose, 33/50; high dose, 15/50; female: 37/50; 37/50; 14/50). Survival of all groups of male mice was low (male: 24/50; 20/50; 14/50; female: 41/50; 38/50; 30/50). Survival of the high dose groups of male mice was significantly lower than that of the vehicle controls; no other significant differences in survival were observed between any groups of mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The incidences of a variety of nonneoplastic lesions were increased in dosed rats of each sex, primarily in the high dose male and female rat groups. These lesions were ulceration and inflammation of the glandular stomach; epithelial hyperplasia of the forestomach; inflammation of the cornea, retinal atrophy, and cataracts of the crystalline lens; focal lipoid degeneration of the adrenal cortex; and diffuse pancreatic atrophy. Cytoplasmic vacuolization and cystic degeneration in the liver occurred at increased incidences in the high dose rat groups; in addition, the incidences of bile duct hyperplasia and bile duct fibrosis were increased in the high dose female and male rat groups, respectively. Bone marrow hematopoietic hyperplasia, hematopoiesis of the spleen, and ultimobranchial cysts of thyroid gland occurred with increased incidences in high dose female rats.

The incidences of thyroid gland follicular cell adenomas or carcinomas (combined) were significantly increased in high dose male (vehicle control, 4/50; low dose, 8/49; high dose, 13/50) and female (2/50; 1/50; 7/50) rats. Follicular cell hyperplasia of the thyroid gland also occurred at an increased incidence in high dose female rats (10/50; 10/50; 26/50) but not in male rats (9/50; 7/49; 7/50). The incidence of pancreatic islet cell adenomas was increased in low dose male rats (0/49; 9/50; 1/49). Adenomas and adenomas or carcinomas (combined) of the anterior pituitary gland occurred at significantly lower incidences in high dose rats than those in vehicle controls (combined incidence—male: 20/47; 14/49; 8/49; female: 18/49; 10/49; 2/48).

Nonneoplastic lesions that occurred at increased incidences in dosed mice included atrophy of the pancreatic acinus and dilatation of the uterus. Depigmentation of hair shafts and change of coat color from agouti to gray were observed in high dose mice. No compound-related neoplasms were observed in dosed mice.

Genetic Toxicology: Malonaldehyde, sodium salt, was not mutagenic in the *Salmonella typhimurium*/microsome assay when tested at doses of up to 10,000 μg/plate in a preincubational protocol using the excision-

repair deficient strains TA98, TA100, TA1535, and TA1537 with or without S9 metabolic activation. The chemical induced forward mutations in mouse L5178Y lymphoma cells in the absence of S9; it was not tested with S9. Malonaldehyde, sodium salt was not mutagenic in the *Drosophila melanogaster* sex-linked recessive lethal mutagenicity test in which adult male flies were exposed either by feeding or by abdominal injection. In cytogenetic assays with cultured Chinese hamster ovary (CHO) cells, malonaldehyde, sodium salt, produced a dose-related increase in the frequency of sister-chromatid exchanges both in the presence and absence of rat liver S9; no increase in the number of chromosomal aberrations was observed in CHO cells in the absence or presence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of malonaldehyde, sodium salt, have been audited. The audit found no special circumstances or significant deficiencies in the conduct or documentation of the studies which needed to be taken into consideration for reporting purposes.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* for male and female F344/N rats administered malonaldehyde, sodium salt, as shown by the increased incidences of follicular cell adenomas or carcinomas (combined) of the thyroid gland. Pancreatic islet cell adenomas were also observed at an increased incidence in low dose male rats. There was *no evidence of carcinogenic activity* for $B6C3F_1$ mice administered 60 or 120 mg/kg malonaldehyde, sodium salt, in distilled water by gavage 5 days per week for 2 years.

Chemically related increased incidences of nonneoplastic lesions included ulcers and inflammation of the glandular stomach and epithelial hyperplasia of the forestomach; corneal inflammation, retinal atrophy, and cataracts of the crystalline lens; and cystic degeneration of the liver, bile duct fibrosis, and bile duct hyperplasia in rats. Most of these nonneoplastic lesions as well as the thyroid gland follicular cell neoplasms occurred primarily in the high dose rat groups, in which survival and final body weights were reduced in high dose male and female rats. Increased incidences of atrophy of the pancreatic acinus and pigmentation loss in hair shafts were seen in high dose mice.

Synonyms: malonaldehyde, enol, sodium salt; propanedial, sodium; 3-hydroxy-2-propenal, sodium salt; sodium β-oxyacrolein

Report Date: November 1988

TR-332 Toxicology and Carcinogenesis Studies of 2-Mercaptobenzothiazole (CAS No. 149-30-4) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of technical-grade 2- mercaptobenzothiazole (96%-97% pure), a rubber accelerant and preservative, were conducted by administering the chemical by gavage in a corn oil vehicle to groups of F344/N rats and $B6C3F_1$ mice of each sex for 16 days, 13 weeks, or 2 years. 2-Mercaptobenzothiazole was nominated for study by the National Institute of Environmental Health Sciences and the National Institute for Occupational Safety and Health.

Sixteen-Day and Thirteen-Week Studies: In 16-day studies, mean body weight gains of rats receiving 2,500 mg/kg were 6-7 g lower than those of vehicle controls; 4/5 male and 5/5 female mice dosed with 3,000 mg/kg and 4/5 female mice dosed with 1,500 mg/kg died; lethargy and prostration occurred in most of these animals after gavage. Based on these results, doses were selected for both species in the 13-week studies were 0, 94 (mice only), 188, 375, 750, and 1,500 mg/kg.

In the 13-week studies, no chemical-related deaths occurred in rats, but body weight gains in males dosed with 1,500 mg/kg and in females dosed with 750 or 1,500 mg/kg were lower than those in the vehicle control groups. Hepatomegaly occurred at the two highest doses in males and at all doses in females; however, no microscopic pathologic changes were noted in any tissue. More than half the mice dosed with 1,500 mg/kg died, but no compound-related body weight changes occurred. Clinical signs in mice were dose related and included lethargy in animals dosed with 375 mg/kg and lacrimation, salivation, and clonic seizure in some dosed with 750 or 1,500 mg/kg. No association between these clinical signs of toxicity and gross or microscopic pathologic effects were observed. Doses selected for the 2-year studies were 0, 375, and 750 mg/kg for male rats and for mice of each sex and 0, 188, or 375 mg/kg for female rats.

Body weight and Survival in the Two-Year Studies: Fifty animals of each species and sex were administered 2-mercaptobenzothiazole in corn oil by gavage 5 days per week for 103 weeks. Administration of 2-mercaptobenzothiazole resulted in decreased survival in dosed male rats (vehicle control, 42/50; low dose, 22/50; high dose, 20/50) and in the high dose group of female mice (37/50; 39/50; 22/50) but not in female rats (28/50; 31/50; 25/50) or in male mice (38/50; 33/50; 30/50). No effect on body weight gain in dosed rats was observed; in dosed mice, minor reductions occurred between weeks 3 and 64, with recovery thereafter. Postgavage lethargy and prostration occurred frequently in dosed rats and mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The severity of nephropathy was increased in dosed male rats. Ulcers and inflammation of the forestomach were prevalent in dosed rats, as were increased incidences of epithelial hyperplasia and hyperkeratosis in male rats, but no neoplasms of the forestomach were observed. There were no increases of nonneoplastic lesions in mice which were considered to be compound related.

The incidences of a variety of tumors were increased in rats dosed with 2-mercaptobenzothiazole; some of the increased incidences were not dose related. In low dose male rats, increased incidences ($P<0.01$) were observed for mononuclear cell leukemia (7/50; 16/50; 3/50) and

pancreatic acinar cell adenomas (2/50; 13/50; 6/49). Increased tumor incidences with dose-related trends ($P<0.05$) included pituitary gland adenomas in females (15/49; 24/50; 25/50), preputial gland adenomas or carcinomas (combined) in males (1/50; 6/50; 5/50), adrenal gland pheochromocytomas or malignant pheochromocytomas (combined) in males (18/50; 27/50; 24/49), and pheochromocytomas in females (1/50; 5/50; 6/50). These tumors were observed at significantly greater incidences ($P \geq 0.05$) in the high dose groups than in the vehicle controls.

An increased incidence ($P = 0.028$) of hepatocellular adenomas or carcinomas (combined) was observed only in low dose female mice (4/50; 12/49; 4/50). No significant increases in tumor incidences were seen in male mice.

Genetic Toxicology: 2-Mercaptobenzothiazole was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation. In the presence of rat liver S9, 2-mercaptobenzothiazole increased the frequency of chromosomal aberrations and sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells, as well as mutations at the TK locus of mouse L5178Y lymphoma cells.

Audit: The data, documents, and pathology materials from the 2-year studies of 2-mercaptobenzothiazole were audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of 2-mercaptobenzothiazole for male F344/N rats, indicated by increased incidences of mononuclear cell leukemia, pancreatic acinar cell adenomas, adrenal gland pheochromocytomas, and preputial gland adenomas or carcinomas (combined). There was *some evidence of carcinogenic activity* for female F344/N rats, indicated by increased incidences of adrenal gland pheochromocytomas and pituitary gland adenomas. There was *no evidence of carcinogenic activity* of 2-mercaptobenzothiazole for male $B6C3F_1$ mice dosed with 375 or 750 mg/kg. There was *equivocal evidence of carcinogenic activity* for female $B6C3F_1$ mice, indicated by increased incidences of hepatocellular adenomas or carcinomas (combined).

Synonyms and Trade Names: Captax; Dermacid; Mertax; Thiotax; 2(3H)-benzothiazolethione; 2-benzothiazolyl mercaptan

Report Date: May 1988

TR-333 Toxicology and Carcinogenesis Studies of *N*-Phenyl-2-naphthylamine (CAS No. 135-88-6) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

N-Phenyl-2-naphthylamine, formerly used as a antioxidant in the rubber industry, was selected for toxicology and carcinogenesis studies because at the time of nomination (1976) it had a large annual production and widespread human exposure. Additional reasons for selection included it structural similarity and possible metabolism to the known human urinary bladder carcinogen, 2-naphthylamine. Toxicology and carcinogenesis studies were conducted by feeding diets containing *N*-phenyl-2-naphthylamine (approximately 98% pure and containing less than 1 ppm 2-naphthylamine) at various concentrations to groups of F344/N rats and $B6C3F_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: In 14-day studies, 3/5 male and 4/5 female rats that received 50,000 ppm N-phenyl-2-naphthylamine died before the end of the studies. Final mean body weights of rats that received 12,500 ppm or more were considerably lower (18%-57%) than those of the controls. Arched backs, rough coats, and diarrhea were observed for males that received 12,500 ppm or more and for females that received 25,000 or 50,000 ppm. All mice were alive at the end of the studies, and no compound-related clinical signs of toxicity were observed in mice given feed containing up to 20,000 ppm.

In 13-week studies, deaths occurred in 4/10 male and 9/10 female rats that received the highest dose (40,000 ppm) of *N*-phenyl-2-naphthylamine. Final mean body weights of rats that received 5,000-40,000 ppm were 9%-60% lower than those of the controls. The liver weight to body weight ratios increased with increasing dose, with the ratios for male rats at 10,000 ppm or more and for female rats at 5,000 ppm being greater ($P<0.05$) than those of controls. A compound-related nephropathy occurred in rats and was characterized by renal tubular epithelial degeneration and hyperplasia. Other effects in rats included hematopoietic hypoplasia or atrophy of the femoral bone marrow, testicular hypospermatogenesis, lymphoid degeneration of the thymus, and lymphoid depletion of the spleen.

In mice, 2/10 males and 7/10 females that received 40,000 ppm died before the end of the 13-week studies. The final mean body weights of mice that received 10,000, 20,000, or 40,000 ppm were 9%-32% lower than those of the controls. The liver weight to body weight ratios for mice increased with increasing dose. Those for male mice at 10,000 ppm or more and for female mice at 20,000 ppm or more were greater ($P<0.05$) than those for the controls. Nephropathy was observed at increased incidences and severity in dosed mice.

Because of kidney lesions, liver enlargement, lower weight gain, and increased mortality in the shorter term studies, dietary concentrations of *N*-phenyl-2-naphthylamine selected for the 2-year studies in rats and mice were 0, 2,5000, and 5,000 ppm.

Body Weight and Survival in the Two-Year Studies: The mean body weights of dosed rats were lower than those of the controls throughout the studies (12% and 16% lower for dosed males and 15% and 31% lower for dosed females at the end of the studies). The average daily feed consumption for rats was 94%-87% that of the controls for dosed males and 88% that of the controls for

dosed females. The estimated average amount of *N*-phenyl-2-naphthylamine consumed per day was 100 mg/kg and 225 mg/kg for male rats and 120 mg/kg and 260 mg/kg for female rats. The survival of the high dose group of male rats was greater ($P<0.05$) than that of the controls after week 101 (male: control, 24/50; low dose, 28/50; high dose, 34/50; female: 26/50; 44/50; 38/50).

Final mean body weights of high dose male and female mice were lower (male, 9%; female, 23%) than those of the controls. The estimated average daily feed consumption by dosed mice was within 10% that of the controls. The average amount of *N*-phenyl-2-naphthylamine consumed per day was approximately 500 or 1,000 mg/kg for male mice and 450 or 900 mg/kg for female mice. No significant differences in survival were observed between any groups of mice of either sex (male: control, 33/50; low dose, 36/50; high dose, 28/50; female: 36/50; 30/50; 35/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: As in the 13-week studies, the kidney was the principal target for toxic effects of N-phenyl-2-naphthylamine. Mineralization of the kidney, necrosis of the renal papilla, and epithelial hyperplasia and calculi of the kidney pelvis were observed at increased incidences in high dose female rats. Hydronephrosis, atrophy, fibrosis, and chronic focal inflammation of the kidney were observed at increased incidences in high dose female rats. Cysts and acute suppurative inflammation of the kidney were observed at increased incidences in dosed male and high dose female rats. No compound-related renal neoplasms were observed in rats.

Nuclear enlargement of renal tubular epithelial cells and nephropathy were observed at increased incidences in high dose female mice. Atypical tubular cell hyperplasia occurred in two high dose female mice. A tubular cell adenoma was found in one high dose female mouse, and a tubular cell adenocarcinoma was found in another high dose female mouse. No renal neoplasms were observed in dosed male mice.

Neoplasms of several organs occurred in rats with negative trends and/or at significantly lower incidences in high dose groups. These included thyroid gland C-cell neoplasms in males and females and mammary gland fibroadenomas, pituitary gland adenomas, and mononuclear cell leukemia in females. The lack of carcinogenicity in rats may be related to an inability to metabolize this compound to the known animal and human carcinogen 2-napththylamine.

Genetic Toxicity: *N*-Phenyl-2-naphthylamine was not mutagenic in the *Salmonella typhimurium*/microsome assay with strains TA97, TA98, TA100, or TA1535 with or without induced hamster or rat liver S9. The chemical did not induce chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells with or without metabolic activation. No increase in sister chromatid exchanges (SCEs) was observed in the absence of metabolic activation; in the presence of rat liver S9, the SCE results were judged to be equivocal.

Data Audit: The data, documents, and pathology materials from the 2-year studies of *N*-phenyl-2-naphthylamine were audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* for male or female F344/N rats fed diets containing 2,500 or 5,000 ppm *N*-phenyl-2-naphthylamine. Decreased incidences of several neoplasms were observed in dosed rats: thyroid gland C-cell neoplasms in males and females and mononuclear cell leukemia, pituitary gland adenomas, and mammary gland fibroadenomas in females. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice fed diets containing 2,500 or 5,000 ppm *N*-phenyl-2-naphthylamine. There was *equivocal evidence of carcinogenic activity* of *N*-phenyl-2-naphthylamine for female $B6C3F_1$ mice as indicated by the occurrence of two rare kidney neoplasms. Chemical-related nonneoplastic lesions (nephropathy, karyomegaly, and hyperplasia) occurred in the kidney of rats and mice.

Synonyms: *N*-(2-naphthyl)aniline; 2-naphthylphenylamine; β-naphthylphenylamine; 2-phenylaminonaphthalene; phenyl-β-naphthylamine; *N*-phenyl-β-naphthylamine

Trade Names: Aceto PBN; Agerite Powder: Antioxidant 116; Neosone D; Neozon D; Nilox PBNA; Nonox D; PBNA; Stabilizator AR

Report Date: January 1988

TR-334 Toxicology and Carcinogenesis Studies of 2-Amino-5-Nitrophenol (CAS No. 121-88-0) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

2-Amino-5-nitrophenol is used as a colorant in semipermanent hair dyes and in the manufacture of C.I. Solvent Red 8, an azo dye for synthetic resins, lacquers, and wood stains. 2-Amino-5-nitrophenol was nominated for toxicology and carcinogenesis studies by the National Cancer Institute because of widespread human exposure associated with its use in hair dyes.

Toxicology and carcinogenesis studies were conducted by administering 2-amino-5-nitrophenol (98% pure) by gavage in corn oil 5 days per week to groups of F344/N rats and $B6C3F_1$ mice of each sex in 16-day, 13-week, and 2-year studies. In the 2-year studies, male and female rats were given doses of 0, 100, or 200 mg/kg and male and female mice were given doses of 0, 400, or 800 mg/kg.

Sixteen-Day and Thirteen-Week Studies: During the 16-day studies, F344/N rats of each sex received 0, 156, 313, 625, 1,250, or 2,500 mg/kg 2-amino-5-nitrophenol by gavage in corn oil vehicle. One of the five males that received 2,500 mg/kg, 1/5 females that received 1,250 mg/kg, and 2/5 females that received 313 mg/kg died before the end of the studies. Final mean body weights of rats that received 1,250 or 2,500 mg/kg were 11% and 30%

lower than that of vehicle controls for males and 9% and 13% lower for females. $B6C3F_1$ mice of each sex received doses of 0, 313, 625, 1,250, 2,500, or 5,000 mg/kg 2-amino-5-nitrophenol. Two of five males and 5/5 females that received 5,000 mg/kg, 3/5 males and 3/5 females that received 2,500 mg/kg, 3/5 females that received 1,250 mg/kg, 1/5 females that received 625 mg/kg, and 2/5 male vehicle controls died before the end of the studies. Final mean body weights of chemically exposed mice were not different from those of the vehicle controls. Rats that received 625, 1,250, or 2,500 mg/kg and male mice that received 5,000 mg/kg had loose stools.

In 13-week studies, F344/N rats and $B6C3F_1$ mice of both sexes received 0, 100, 200, 400, 800, or 1,600 mg/kg 2-amino-5-nitrophenol by gavage in corn oil. Five of 10 male and 2/10 female rats that received 1,600 mg/kg, 1/10 male and 3/10 female rats that received 800 mg/kg, and 1/10 male rats that received 400 mg/kg died before the end of the studies. Final mean body weights of males that received 400, 800, or 1,600 mg/kg were 10%, 25%, and 43% lower than that of vehicle controls. The final mean body weight of females that received 1,600 mg/kg was 16% lower that of vehicle controls.

Four of 10 male and 3/10 female mice that received 1,600 mg/kg died before the end of the 13-week studies. The final mean body weight of male mice that received 1,600 mg/kg was 11% lower than that of vehicle controls; male and female mice that received 1,600 mg/kg appeared lethargic.

During the 13-week studies, acute/chronic perivasculitis of vessels of the cecum and colon was observed in rats that received 400, 800, or 1,600 mg/kg and in mice that received 1,600 mg/kg.

Body Weight and Survival in the Two-Year Studies: Mean body weights of rats receiving 200 mg/kg were 5%-10% lower than those of vehicle controls after week 33 for males and 4%-5% lower than those of vehicle controls after week 93 for females. Survival of male rats was significantly lower than that of vehicle controls after week 99 for the 100 mg/kg dose group and after week 75 for the 200 mg/kg dose group (final survival: vehicle control, 33/50; 100 mg/kg group, 16/50; 200 mg/kg group, 4/50). Survival of female rats was comparable to that of vehicle controls (30/50; 32/50; 29/50). Loose or poorly formed stools were observed for male rats and occasionally for females that received 200 mg/kg.

Mean body weights of mice that received 800 mg/kg were 8%-11% lower than those of vehicle controls between weeks 29 and 74 for males and 8%-13% lower than those of vehicle controls after week 69 for females; mean body weights of mice that received 400 mg/kg were greater than those of vehicle controls after week 69 for males and 5%-9% lower than those of vehicle controls after week 69 for females. Survival of mice that received 800 mg/kg was significantly reduced compared with that of vehicle controls after week 20 for males and week 22 for females and was not considered adequate to evaluate a carcinogenic response (final survival—male: vehicle control, 31/50; 400 mg/kg group, 36/50; 800 mg/kg group, 12/50; female: 37/50; 36/50; 10/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Pigmentation was present at increased incidences in all groups of chemically exposed animals and was characterized by varying amounts of an orange, granular pigment present in the fibrous connective tissue of the lamina propria, in the submucosa, and around vessels in the submucosa of the cecum and colon. Pigmentation of the rectum was observed at increased incidences in male rats that received 100 mg/kg, male and female rats that received 200 mg/kg, and both groups of chemically exposed mice.

No pigmentation was found in the intestines of vehicle control rats or mice. Associated with pigmentation was an increased incidence of acute/chronic inflammation in the cecum and colon of all groups chemically exposed rats and mice; this inflammation was similar to that observed in the 13-week studies but was of greater severity. Acute/chronic inflammation was also present in the rectum of male rats that received 100 mg/kg, male and female rats that received 200 mg/kg, and male mice that received 800 mg/kg.

The incidence of pancreatic acinar cell adenomas was significantly increased ($P \leq 0.002$) in male rats that received 100 mg/kg 2-amino-5-nitrophenol (vehicle control, 1/50; 100 mg/kg, 10/50; 200 mg/kg, 3/49); the increase was considered to be associated with chemical exposure. The reduced survival of male rats that received 200 mg/kg markedly reduced the sensitivity of this group for detecting the presence of neoplasms. The incidences of adenomas or carcinomas (combined) of the preputial or clitoral glands were marginally increased in male or female rats that received 200 mg/kg 2-amino-5-nitrophenol (preputial gland: 3/50; 2/50; 5/50; clitoral gland: 3/50; 3/50; 7/50). Neoplasms found in the intestinal tract of 3/50 male rats that received 100 mg/kg (one leiomyoma of the small intestine, one adenocarcinoma of the jejunum, one leiomyoma of the cecum), 2/50 male rats that received 200 mg/kg (one lipoma and one osteosarcoma of the cecum), and 1/50 female rats that received 200 mg/kg (one leiomyoma of the cecum) were not considered to be the result of chemical exposure. No compound-related neoplasms were found in mice exposed to 2-amino-5-nitrophenol in the 2-year studies.

Genetic Toxicology: 2-Amino-5-nitrophenol was mutagenic in *Salmonella typhimurium* strains TA98, TA100, and TA1537 when tested in a preincubation protocol with and without exogenous metabolic activation, and it exhibited equivocal mutagenic activity in strain TA1535 in the presence of induced liver S9. 2-Amino-5-nitrophenol induced forward mutations in mouse L5178Y lymphoma cells in the absence of metabolic activation; it was not tested with S9. An increase in chromosomal aberrations and sister chromatid exchanges was observed in cultured Chinese hamster ovary (CHO) cells following incubation with 2-amino-5-nitrophenol both in the presence and absence of exogenous metabolic activation.

Data Audit: The data, documents, and pathology materials from the 2-year studies of 2-amino-5-nitrophenol were audited at the NTP Archives. The audit findings show that the conduct of the studies is documented

adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* for male F344/N rats that received 100 mg/kg 2-amino-5-nitrophenol, as shown by the increased incidence of acinar cell adenomas of the pancreas. Reduced survival of male F344/N rats that received 200 mg/kg decreased the sensitivity of this group for detecting a carcinogenic response. There was *no evidence of carcinogenic activity* for female rats that received 100 or 200 mg/kg per day. Marginally increased incidences of preputial or clitoral gland adenomas or carcinomas (combined) occurred in male and female F344/N rats administered 200 mg/kg 2-amino-5-nitrophenol. There was *no evidence of carcinogenic activity* for $B6C3F_1$ mice that received 400 mg/kg 2-amino-5-nitrophenol; reduced survival of $B6C3F_1$ mice that received 800 mg/kg caused this group to be considered inadequate for detecting a carcinogenic response.

Report Date: February 1988

TR-335 Toxicology and Carcinogenesis Studies of C.I. Acid Orange 3 (CAS No. 6373-74-6) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

C.I. Acid Orange 3 is a dinitrodiphenylamine derivative used exclusively as a dye (up to 0.2%) in semipermanent hair coloring products. This study was one of a series on semipermanent hair dyes, which included HC Blue No. 1 (NTP TR 271), HC Blue No. 2 (NTP TR 293), HC Red No. 3 (NTP TR 281), and C.I. Disperse Blue 1 (NTP TR 299). Toxicology and carcinogenesis studies of C.I. Acid Orange 3 (90% pure, containing 10% water for short-term studies and containing 6%-8% water and 2%-4% acetone for 2-year studies) were conducted by administering the dye in corn oil by gavage to F344/N rats and $B6C3F_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies (at 94-1,500 mg/kg in rats and 62-1,000 mg/kg in mice), no compound-related deaths or body weight changes were observed and no adverse effects were observed at necropsy.

In the 13-week studies (at 94-1,500 mg/kg in rats and 31-2,000 mg/kg in mice), compound-related kidney lesions were observed in rats and mice of each sex. These lesions included variable degrees of degeneration and necrosis of epithelial cells in the proximal convoluted tubules, regeneration of tubular epithelium, and granular casts in the tubules. In a few female rats of the highest dose group, necrosis of the renal papillae and suppurative inflammation were also observed. Mean body weights were generally comparable among groups of rats and mice. Mice receiving 2,000 mg/kg had body weights 11%-12% lower than those of vehicle controls. Five of 10 female rats that received the highest dose of 1,500 mg/kg died before the end of the study, but no compound-related deaths occurred in male rats or mice of either sex.

Based on these results, 2-year studies of C.I. Acid Orange 3 were conducted by administering the dye by gavage in corn oil at 0, 375, or 750 mg/kg to groups of 50 F344/N rats of each sex, 5 days per week for 103 weeks. Groups of 50 male $B6C3F_1$ mice were administered 0, 125, or 250 mg/kg C.I. Acid Orange 3 on the same schedule, and groups of 50 female $B6C3F_1$ mice were administered 0, 250, or 500 mg/kg. These doses were selected on the basis of the nature and severity of the renal lesions in both species.

Body Weights and Survival in the Two-Year Studies: Mean body weights of high dose rats were generally more than 10% lower than those of vehicle controls after week 52 for males and week 70 for females. Mean body weights for low dose groups were comparable to those of vehicle controls. The survival of high dose male (after week 33) and female (after week 14) rats was lower ($P<0.05$) than that of vehicle controls and was attributed to nephrotoxicity (final survival—male: vehicle control, 36/50; low dose, 30/50; high dose, 0/50; female: 43/50; 34/50; 7/50). Mean body weights of dosed male and female mice were lower than those of vehicle controls (high dose, 5%-11% after week 74; low dose, 7%-17% after week 48). Survival of both the low dose (after week 102) and high dose (after week 100) groups of male mice was lower than that of the vehicle controls (final survival: 38/50; 25/50; 26/50). Although survival was lower than usual, no notable differences in survival were observed between groups of female mice (final survival: 23/50; 23/50; 24/50).

Nonneoplastic and Neoplastic Lesions in the Two-Year Studies: For both species, the kidney was the major target organ for C.I. Acid Orange 3. These findings are summarized in the accompanying table. The incidences of renal pelvic epithelial hyperplasia were increased in dosed rats of each sex. No renal neoplasms were observed in dosed male rats, but a tubular cell adenocarcinoma was observed in a vehicle control male rat. Six transitional cell carcinomas of the kidney were observed in high dose female rats; kidney transitional cell neoplasms have not been observed in 1,697 corn oil vehicle control female F344/N rats.

Nonneoplastic lesions characteristic of secondary renal hyperparathyroidism or secondary to uremia also occurred in dosed rats. The lesions included parathyroid hyperplasia, fibrous dysplasia of bone, erosion and ulcers of the glandular stomach, and mineralization of the aorta and glandular stomach.

Epithelial hyperplasia of the urinary bladder was observed in one low dose and three high dose female mice. A squamous cell carcinoma was seen in the urinary bladder of one low dose female mouse. Even though no squamous cell urinary bladder neoplasms have been observed in 1,665 corn oil vehicle control female $B6C3F_1$ mice, this single neoplasm in a low dose animal was not considered to be related to the administration of C.I. Acid Orange 3.

Genetic Toxicology: C.I. Acid Orange 3 was mutagenic with and without exogenous metabolic activation in *Salmonella typhimurium* strains TA97; TA98; and TA100; no mutagenicity was observed for strain TA1535.

Audit: The data, documents, and pathology materials from the 2-year studies of C.I. Acid Orange 3 have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of C.I. Acid Orange 3 for male F344/N rats administered 375 mg/kg; because of a marked reduction in survival and no indication of carcinogenicity, the 750 mg/kg group was considered to be inadequate for assessment of carcinogenic activity. There was *clear evidence of carcinogenic activity* of C.I. Acid Orange 3 for female F344/N rats as shown by the occurrence of transitional cell carcinomas of the kidney in the 750 mg/kg group; this group had reduced survival and chemically related nonneoplastic lesions of the kidney. There was *no evidence of carcinogenic activity* of C.I. Acid Orange 3 for male $B6C3F_1$ mice administered 125 or 250 mg/kg or for female $B6C3F_1$ mice administered 250 or 500 mg/kg. Nonneoplastic lesions of the kidney were observed in both dose groups of both sexes of rats and mice.

Synonyms: 2-anilino-5-(2,4-dinitroanilino)-benzenesulfonic acid, monosodium salt; 5[(2,4-dinitrophenol)-amine]-2-(phenylamine)-benzenesulfonic acid, monosodium salt; C.I. 10385; Tetracid Light Yellow 2R

Report Date: December 1988

TR-336 Toxicology and Carcinogenesis Studies of Penicillin VK (CAS No. 132-98-9) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Penicillin VK, a widely used antibiotic for treatment of gram-positive coccal infections, was nominated for study by the National Cancer Institute because rodent carcinogenicity studies for this drug had not been performed. The chemical (94% or 98% pure, USP grade) was administered orally (by gavage in corn oil) because oral administration is the primary route used to treat infections in humans. Fourteen-day, 13-week, and 2-year studies were conducted in F344/N rats and $B6C3F_1$ mice. Additional studies were performed to evaluate the potential for genetic damage in bacteria and mammalian cells.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, penicillin VK was administered at doses of 150-2,400 mg/kg. No compound-related deaths or dose-related histopathologic lesions were seen in rats or mice. Final mean body weights of dosed male rats were 5%-17% lower than that of controls; weights of dosed and control female rats were comparable. Final mean body weights of dosed mice were 5%-9% lower than those of controls. Diarrhea was observed in all dosed groups of rats and mice.

In the 13-week studies, male and female rats received doses of 180-3,000 mg/kg and male and female mice received doses of 250-3,000 mg/kg. No compound-related deaths were seen in rats or mice. Final mean body weights of rats that received 3,000 mg/kg were 11% lower than those of the vehicle controls for males and 6% lower for females. For mice, mean body weights were comparable. Diarrhea occurred in male rats at doses of 750 mg/kg and above and in female rats at doses of 1,500 and 3,000 mg/kg. Mucous cell metaplasia of the glandular stomach was observed in male and female rats receiving 1,500 and 3,000 mg/kg. Lesions of the glandular stomach (inflammation, mucous cell metaplasia, and eosinophilic cytoplasmic change) and the forestomach (papillary hyperplasia and hyperkeratosis) were seen in all groups of dosed mice. The severity of lesions at 1,000 mg/kg or below was considered minimal. Based on these results, doses selected for rats and mice in the 2-year studies were 0, 500, or 1,000 mg/kg.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed and vehicle control male and female rats and male mice were comparable. Mean body weights of dosed female mice were 4%-16% lower than those of the vehicle controls from week 28 to the end of the study. Diarrhea was observed for dosed male and female rats and for dosed male mice. Survival of low and high dose male rats and high dose female rats was reduced (male rats: vehicle control, 34/50; low dose, 19/50; high dose, 16/50; female rats: 29/50; 26/50; 16/50). Survival of male and female mice was comparable to that of the vehicle controls (male mice: 24/50; 36/50; 26/50; female mice: 36/50; 32/50; 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nonneoplastic lesions occurred at low incidences in the nasal mucosa, lung, and forestomach of dosed male rats and in the nasal mucosa and lung of dosed female rats. Congestion and aspiration pneumonia occurring in dosed rats dying before week 104 was the principal cause of death in these animals.

Nonneoplastic lesions of the gastric fundal gland (eosinophilic cytoplasmic change and dilatation) and glandular stomach (cyst, chronic focal inflammation, hyperplasia, fibrosis, and squamous metaplasia) were seen in dosed male and female mice, and lesions of the gallbladder (eosinophilic cytoplasmic change) were seen in male mice.

Slight increases in the incidences of adenomas of the pituitary gland in high dose male rats and of fibroadenomas or adenomas (combined) of the mammary gland in low dose female rats were observed. These were not considered to be compound-related lesions.

The incidence of hepatocellular adenomas was decreased in high dose male mice (14/50; 15/49; 4/49). No compound-related neoplasms were seen in female mice.

Genetic Toxicology: Penicillin VK was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation. The chemical was mutagenic only with activation in the mouse lymphoma L5178Y/$TK^{+/-}$ forward mutation

assay. Incubation of Chinese hamster ovary cells with penicillin VK resulted in increased frequencies of sister chromatid exchanges and chromosomal aberrations in the absence of metabolic activation under the conditions of delayed harvest to compensate for chemical-induced cell cycle delay, no effects from penicillin VK exposure were observed in these cells in the presence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of penicillin VK were audited. The audit findings show that the conduct of the studies is documented and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of penicillin VK for F344/N rats or for $B6C3F_1$ mice administered 500 or 1,000 mg/kg penicillin VK in corn oil gavage, 5 days per week for 2 years. Nonneoplastic lesions were seen in the glandular stomach of dosed mice. Decreased survival of low and high dose male rats and of high dose female rats reduced the sensitivity of the studies for determining the presence or absence of a carcinogenic response in this species.

Synonyms: 4-thia-1-azabicyclo(3.2.0)heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-(2-phenoxyacetamide)-, monopotassium salt; penicillin V potassium; penicillin V potassium salt; D-α-phenoxymethylpenicillinate K salt; phenoxymethylpenicillin potassium; PVK

Trade Names: Antibiocin; Apsin VK; Aracil; Arcasin; Aspin VK; Beromycin; Beromycin 400; Betapen VK; Calciopen K; Cliacil; Compocillin VK; Distakaps V-K; Distaquaine V-K; Dowpen V-K; DQV-K; Fenoxypen; Icipen; Isocillin; Ispenoral; Ledercillin VK; Megacillin oral; Oracil-VK; Orapen; Ospeneff; Pedipen; Penagen; Pencompren; Pen-Vee K; Pen-V-K powder; Penvikal; Pfizerpen VK; Qidpen VK; Robicillin VK; Rocillin-VK; Roscopenin; SK-Penicillin VK; Stabilin VK Syrup 125; Stabilin VK Syrup 62.5; Sumapen VK; Suspen; Uticillin VK; V-Cil-K; V-Cillin K; Veetids; Vepen

Report Date: June 1988

TR-337 Toxicology and Carcinogenesis Studies of Nitrofurazone (CAS No. 59-87-0) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Nitrofurazone is a synthetic furan derivative, active against a broad spectrum of bacteria, which has been widely used in veterinary and human medicine. Toxicology and carcinogenesis studies were conducted by feeding diets containing nitrofurazone (99% pure) to groups of F344/N rats and $B6C3F_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: Groups of five males and five females of each species were fed diets containing 0, 630, 1,250, 2,500, 5,000, or 10,000 ppm for 14 consecutive days. Early deaths occurred in all groups of rats receiving 5,000 or 10,000 ppm nitrofurazone. The surviving rats in the lower two dose groups gained weight, but weight gain was decreased as the dose of nitrofurazone was increased. Feed consumption by rats of each sex was decreased at all doses above 630 ppm. In all dosed groups, clinical signs of toxicity included rough hair coats and lethargy. At doses of 2,500 ppm and above, rats of each sex exhibited intermittent episodes of seizures and lethargy.

All mice that received 2,500, 5,000, or 10,000 ppm nitrofurazone and 3/5 males that received 1,250 ppm died before the end of the 14-day studies; the surviving dosed mice (except females at 630 ppm) lost weight. A dose-related decrease in feed consumption was observed at all doses above 630 ppm. Clinical signs included rough hair coats and convulsive seizures.

In the 13-week studies, groups of 10 rats of each sex were given diets containing 0, 150, 310, 620, 1,250, or 2,500 ppm nitrofurazone. No deaths were observed and all animals gained weight, but the magnitude of weight gain was dose dependent with decrements in final mean body weight for the highest dose group reaching 55% in males and 36% in females. Other evidence of chemically related toxicity included convulsive seizures, osteoporosis, degenerative arthropathy, and gonadal hypoplasia in both sexes at the two highest doses.

Groups of 10 mice of each sex were given diets containing 0, 70, 150, 310, 620, or 1,250 ppm nitrofurazone for 13 weeks. Early deaths were observed in the two highest dose groups of each sex. The final mean body weights of male and female mice in the 1,250-ppm groups were about 20% lower than those of the controls; weight gains of the other dosed mice were comparable to those of the controls. Stimulus-induced convulsive seizures were observed for all mice in the two highest dose groups. Testicular hypoplasia was observed in the two highest dose groups of male mice.

Body Weight and Survival in the Two-Year Studies: Dietary concentrations for the 2-year studies were 0, 310, or 620 ppm for rats and 0, 150, or 310 ppm for mice (50 animals per dose group). Mean body weights of high dose male rats were lower than those of the controls after week 39; mean body weights of low dose male rats and of the controls were comparable throughout the study. Final mean body weights of low and high dose female rats were 9% and 21% lower than those of the controls. Dosed rats consumed less feed than did the controls. The average amount of nitrofurazone consumed per day was approximately 11-12 or 24-26 mg/kg by low or high dose male and female rats. The survival of the high dose group of male rats was lower than that of the controls after week 92 (final survival—male: control, 33/50; low dose, 30/50; high dose, 20/50; female: 28/50; 37/50; 31/50).

Mean body weights of dosed mice were similar to or somewhat greater than those of controls throughout most of the studies. The average daily feed consumption by dosed mice was similar to that of controls. The average amount of nitrofurazone consumed per day was approximately 14-16 or 29-33 mg/kg for low or high dose male and female mice. The survival of the high dose group of male mice was lower than that of the controls after week

88 (final survival—male: 39/50; 31/50; 27/50; female: 39/50; 40/50; 35/50).

In mice of each sex, nitrofurazone administration induced stimulus-sensitive convulsive seizures beginning at week 4 or 5 for high dose mice and week 24 for low dose female mice. These seizures were observed primarily in the first year of the study.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Degenerative changes involving the vertebral and femoro-tibial (knee) joints were observed at increased incidences in dosed rats. The degenerative changes primarily affected the articular cartilage and were similar to those seen in the 13-week studies. Degeneration of the sternal synchondroses was increased in high dose female rats. The osteoporosis seen in the 13-week studies was not observed in the 2-year studies. Testicular degeneration, characterized by atrophy of the germinal epithelium and aspermatogenesis, was observed at increased incidences in dosed male rats (control, 12/50; low dose, 49/50; high dose, 47/50).

Adenomas of the sebaceous glands and trichoepitheliomas or sebaceous adenomas (combined) of the skin were observed in high dose male rats (0/50; 0/50; 5/50). Carcinomas of the preputial gland were increased in dosed male rats (1/50; 8/50; 5/50). The incidences of preputial gland adenomas or carcinomas (combined) in dosed male rats were not statistically greater than that in the controls (9/50; 16/50; 7/50). However, in the low dose group, the incidence is greater than the highest incidence observed in historical untreated control groups (9/50). In addition, hyperplasia of the preputial gland was observed in six low dose male rats in which neither adenomas nor carcinomas occurred. The incidence of mesotheliomas of the tunica vaginalis in low dose male rats was greater than that in the controls (0/50; 7/50; 2/50).

Fibroadenomas of the mammary gland occurred at markedly increased incidences in dosed female rats (8/49; 36/50; 36/50). Three adenocarcinomas were also observed (1/49; 0/50; 2/50).

Ovarian atrophy (7/47; 44/50; 38/50) and tubular cell hyperplasia of the ovary (1/47; 23/50; 21/50) were observed at markedly increased incidences in dosed female mice. The incidences of benign mixed tumors (0/47; 17/50; 20/50), granulosa cell tumors (1/47; 4/50; 9/50), and granulosa cell tumors or luteomas (combined) (3/47; 6/50; 9/50) of the ovary were increased in exposed female mice.

Mononuclear cell leukemia in rats occurred with negative trends (male: 21/50; 23/50; 6/50; female: 15/49; 2/50; 2/50). In female mice, the incidences of adenomas or carcinomas (combined) of the anterior pituitary gland occurred with a negative trend (10/50; 7/50; 2/49). The incidences of testicular interstitial cell tumors were decreased in dosed male rats (45/50; 30/50; 28/50).

Genetic Toxicity: Nitrofurazone was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 both with and without exogenous metabolic activation. The responses in strains TA1535 and TA1537 were more varied: nitrofurazone was mutagenic in strain TA1535 only in the presence of S9 and produced no consistent increase in gene reversions in strain TA1537 with or without S9. In the absence of metabolic activation, nitrofurazone induced forward mutations at the $TK^{+/-}$ locus of mouse L5178Y lymphoma cells; the chemical was not tested with S9. Treatment of cultured Chinese hamster ovary cells with nitrofurazone in the absence of S9 produced a dose-related increase in sister chromatid exchanges and chromosomal aberrations; with S9, sister chromatid exchanges were increased, but no induction of chromosomal aberrations was observed.

Audit: The data, documents, and pathology materials from the 2-year studies of nitrofurazone were audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of nitrofurazone for male F344/N rats as shown by the occurrence of sebaceous gland adenomas and trichoepitheliomas of the skin, mesotheliomas of the tunica vaginalis, and preputial gland tumors. There was *clear evidence of carcinogenic activity* of nitrofurazone for female F344/N rats as shown by a markedly increased incidence of fibroadenomas of the mammary gland. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice fed diets containing nitrofurazone at concentrations of 150 or 310 ppm. There was *clear evidence of carcinogenic activity* of nitrofurazone for female $B6C3F_1$ mice as shown by increased incidences of benign mixed tumors and granulosa cell tumors of the ovary.

Administration of nitrofurazone was associated with decreased incidences of mononuclear cell leukemia in male and female rats, testicular interstitial cell tumors in male rats, and pituitary gland neoplasms in female mice. Convulsive seizures in mice of each sex, ovarian atrophy in female mice, testicular degeneration in rats, and degeneration of articular cartilage in rats were all associated with the administration of nitrofurazone.

Synonyms: 5-nitro-2-furaldehyde semicarbazone; 2-[(5-nitro-2-furanyl)methylene]hydrazine carboximide

Trade Names: Aldomycin; Amifur; Chemfuran; Coxistat; Furacin; Furacinetten; Furaplast; Furazol W; Furesol; Furracoccid; Mammex; Nefco; Nifuzon; Nitrofural; Vabrocid

Report Date: June 1988

TR-338 Toxicology and Carcinogenesis Studies of Erythromycin Stearate (CAS No. 643-22-1) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies) TR-338

Toxicology and carcinogenesis studies of erythromycin stearate (USP grade, greater than 96% pure) were conducted by administering the antibiotic in feed to groups

of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Erythromycin stearate was studied because of its widespread use in humans as a broad-spectrum macrolide antibiotic and because of the lack of adequate long-term studies for carcinogenicity.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, none of the rats (at dietary concentrations up to 50,000 ppm) and 2/5 female mice that received 50,000 ppm died before the end of the studies. Final mean body weights of male rats that received 12,500, 25,000, or 50,000 ppm were 10%, 30%, or 36% lower, respectively, than that of controls; final mean body weights of female rats were 10%, 12%, or 32% lower. None of the dosed mouse groups gained weight. The final mean body weight of male mice that received 50,000 ppm was 10% lower than that of controls.

In the 13-week studies, none of the rats or mice (at dietary concentrations up to 20,000 ppm) died before the end of the studies. Final mean body weights of the 20,000-ppm groups of rats were more than 12% lower than that of the controls for males and 7% lower for females. Final mean body weights of mice that received 10,000 or 20,000 ppm were 15% or 19% lower than that of controls for males and 5% or 14% lower for females.

Multinucleated syncytial hepatocytes were observed in 10/10 male rats that received 20,000 ppm but in 0/10 male rats that received 10,000 ppm. No compound-related gross or microscopic pathologic effects were observed in mice.

Based on these results, 2-year studies of erythromycin stearate were conducted by feeding diets containing 0, 5,000, or 10,000 ppm erythromycin stearate to groups of 50 rats of each sex for 103 weeks. Diets containing 0, 2,500, or 5,000 ppm were fed to groups of 50 mice of each sex for 103 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male rats were comparable to those of controls throughout the studies. Mean body weights of high dose female rats were 5%-10% lower than those of controls. Mean body weights of dosed and control mice were comparable. The average daily feed consumption was similar for dosed and control male and female rats. For mice, estimated daily feed consumption by low and high dose males was similar to that of the controls and by low and high dose females was 92% that of the controls. The average amount of erythromycin stearate consumed per day was approximately 180 or 370 mg/kg for male rats and 210 or 435 mg/kg for female rats; for mice, the average amounts were 270 or 545 mg/kg for males and 250 or 500 mg/kg for females.

No significant differences in survival were observed between any groups of rats or mice of either sex (final survival—male rats: control, 28/50; low dose, 23/50; high dose, 27/50; female rats: 29/50; 30/50; 38/50; male mice: 34/50; 33/50; 40/50; female mice: 38/50; 34/50; 40/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Granulomas of the liver were observed at increased incidences in high dose rats (male: 1/50; 2/50; 10/50; female: 18/50; 27/50; 43/50). Granulomatous inflammation or granulomas of the spleen were observed in dosed female rats (0/48; 1/49; 3/50). Reticulum cell hyperplasia in the bone marrow occurred at increased incidences in high dose female rats (10/50; 14/50; 25/50).

Squamous cell papillomas of the oral mucosa were observed in 1/50 control, 2/50 low dose, and 3/50 high dose female rats. These tumors were considered to be marginal and not related to exposure. Hyperplasia of the oral mucosa was not observed.

Pheochromocytomas of the adrenal gland in female rats occurred with a positive trend (1/50; 4/49; 5/50). The incidences in the dosed groups are similar to the average historical incidence (9%) of this tumor in untreated control female F344/N rats at the study laboratory. This marginal tumor increase is not considered to be biologically important. No increases in incidences of neoplasms were observed at any site in dosed male rats.

Inflammation in the glandular stomach was observed at increased incidences in dosed male mice (1/49; 4/50; 6/50). Lymphoid hyperplasia in the urinary bladder was observed at increased incidences in dosed female mice (1/50; 9/47; 7/48).

No increases in incidences of neoplasms were observed at any site in dosed male or female mice.

Genetic Toxicology: Erythromycin stearate was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested both with or without exogenous metabolic activation. Erythromycin stearate demonstrated equivocal mutagenicity in the mouse L5178Y lymphoma cell assay in the absence of exogenous metabolic activation (S9); erythromycin stearate was not mutagenic in the presence of S9. Treatment of cultured Chinese hamster ovary cells with erythromycin stearate did not produce an increase in the frequency of sister chromatid exchanges or chromosomal aberrations in either the presence or absence of metabolic activation.

Audit: The data, documents, and pathology materials from the 2-year studies of erythromycin stearate have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year studies, there was *no evidence of carcinogenic activity* of erythromycin stearate for male or female F344/N rats administered erythromycin stearate in the diet at 5,000 or 10,000 ppm. There was *no evidence of carcinogenic activity* of erythromycin stearate for male or female B6C3F$_1$ mice administered erythromycin stearate in the diet at 2,500 or 5,000 ppm. Dose-related increases in the incidences of granulomas of the liver were observed in male and female rats. The absence of any biologically important chemical-associated effects in mice suggests that higher doses could have been given to male and female mice.

Synonyms: erythrocin stearate; erythromycin octadecanoate

Trade Names: Abbotcine; Bristamycin; Dowmycin E; Eratrex; Erypar; Ethril; Gallimycin; HSDB 4178; OE 7; Pantomicina; Pfizer-E; SK-Erythromycin; Wyamycin S

Report Date: December 1988

TR-339 Toxicology and Carcinogenesis Studies of 2-Amino-4-Nitrophenol (CAS No. 99-57-0) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

2-Amino-4-nitrophenol is used to color semipermanent hair dyes and in the manufacture of mordant dyes for leather, nylon, silk, wool, and fur. 2-Amino-4-nitrophenol was nominated by the National Cancer Institute for toxicology and carcinogenesis studies because of widespread human exposure associated with its manufacture and use. Toxicology and carcinogenesis studies were conducted by administering 2-amino-4-nitrophenol (98% pure) in corn oil by gavage, 5 days per week, to groups of F344/N rats and B6C3F$_1$ mice of each sex in 15-day, 13-week, and 2-year studies.

Fifteen-Day and Thirteen-Week Studies: During the 15-day studies, rats and mice received doses of 0, 313, 625, 1,250, 2,500, or 5,000 mg/kg. All rats that received 2,500 or 5,000 mg/kg and all female rats that received 1,250 mg/kg died before the end of the studies. Final mean body weights of chemically exposed rats surviving to the end of the studies were comparable to those of vehicle controls. Diarrhea was observed in all groups of exposed rats except those receiving 313 mg/kg. All mice that received 2,500 or 5,000 mg/kg, 2/5 males and all females that received 1,250 mg/kg, and 1/5 females that received 313 mg/kg died before the end of the studies. Final mean body weights of exposed mice surviving until the end of the studies were comparable to those of vehicle controls.

In 13-week studies, F344/N rats and B6C3F$_1$ mice of each sex received 2-amino-4-nitrophenol at doses of 0, 62.5, 125, 250, 500, or 1,000 mg/kg. All rats that received 1,000 mg/kg and 2/10 males and 2/10 females that received 500 mg/kg died before the end of the studies. The final mean body weight of male rats that received 500 mg/kg was reduced 10% compared with that of vehicle controls; final mean body weights of all other surviving exposed rat groups were comparable to those of vehicle controls. Diarrhea and lethargy were observed for rats that received 500 or 1,000 mg/kg. All male mice and most females that received 1,000 mg/kg and 4/10 females that received 500 mg/kg died before the end of the studies. Final mean body weights of chemically exposed mice were comparable to those of vehicle controls. No compound-related clinical signs were observed in mice during the studies.

Mineralization of the renal cortex and degeneration of the renal tubular epithelium were observed in male and female rats that received 1,000 mg/kg and in males that received 500 mg/kg. Degeneration and necrosis of the renal tubular epithelium was observed in 5/10 male and 3/10 female mice that received 1,000 mg/kg.

Body Weight and Survival in the Two-Year Studies: In the 2-year studies, rats and mice received 2-amino-4-nitrophenol at doses of 0, 125, or 250 mg/kg. Mean body weights of male rats that received 250 mg/kg were 8%-10% lower than those of vehicle controls throughout most of the 2-year study. Mean body weights of female rats were comparable to those of vehicle controls. Soft stools and occasional diarrhea were observed in chemically exposed rats starting 6 months after the beginning of the studies. Survival of male rats that received 250 mg/kg was markedly lower than that of vehicle controls after week 89 (final survival: vehicle control, 32/50; 125 mg/kg group, 24/50; 250 mg/kg group, 10/50). Survival of female rats was comparable among all groups (final survival: 25/50; 27/50; 31/50).

Mean body weights of male and female mice that received 250 mg/kg were comparable to those of vehicle controls; the mean body weights of female mice that received 125 mg/kg were as much as 17% greater than that of vehicle controls. Survival of all mouse groups was comparable during the 2-year studies (final survival: male – 28/50; 29/50; 23/50; female – 28/50; 31/50; 30/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Pigmentation of the small and large intestines was present in exposed rats but not in vehicle controls. Ulcers and erosive lesions of the digestive tract were observed in male rats that received 250 mg/kg and to a lesser extent in male rats that received 125 mg/kg. A carcinoma of the colon occurred in one male rat that received 250 mg/kg; no other neoplasms were observed in the gastrointestinal tract of rats. No pigmentation, ulcers, or erosive lesions were found in the digestive tract of mice.

The severity of nephropathy was markedly greater in exposed male rats than in vehicle controls. Associated with the nephropathy were nonneoplastic lesions indicative of reduced renal function and secondary hyperparathyroidism, including parathyroid hyperplasia, mineralization of various organs, and fibrous osteodystrophy.

Renal tubular cell hyperplasia (1/50; 4/48; 5/50) and renal cortical (tubular cell) adenomas (0/50; 1/48; 3/50) occurred in male rats. Renal cortical adenomas are infrequently observed in male F344/N rats (historical incidence, 0.5%).

More preputial gland adenomas or carcinomas (combined) were observed in low dose male rats than in vehicle controls (3/50; 10/48; 3/50), whereas the incidences of clitoral gland neoplasms were decreased in dosed female rats (9/50; 6/50; 1/49).

Hemangiomas or hemangiosarcomas (combined) occurred in male mice that received 2-amino-4-nitrophenol (0/50; 1/50; 5/50); each tumor was present at a different site. The historical control incidence is 11% at the study laboratory and 6% in 2-year NTP studies.

Genetic Toxicology: 2-Amino-4-nitrophenol was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 with metabolic activation. 2-Amino-4-nitrophenol was not mutagenic in strains TA1535 or TA1537. 2-Amino-4-nitrophenol was mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay without metabolic activation. It was not tested with activation. 2-Amino-4-

nitrophenol induced sister chromatid exchanges (SCEs) and chromosomal aberrations in Chinese hamster ovary cells in the presence and absence of metabolic activation.

Audit: The data, documents, and pathology materials from the 2-year studies of 2-amino-4-nitrophenol were audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of 2-amino-4-nitrophenol for male F344/N rats, as shown by increased incidences of renal cortical (tubular cell) adenomas. The incidences of renal tubular cell hyperplasia were also increased in male rats exposed to 2-amino-4-nitrophenol. The survival of male rats that received 2-amino-4-nitrophenol was reduced compared with survival of vehicle control male rats. There was *no evidence of carcinogenic activity* of 2-amino-4-nitrophenol for female F344/N rats or for male or female $B6C3F_1$ mice that received 125 or 250 mg/kg per day.

Report Date: June 1988

TR-340 Toxicology and Carcinogenesis Studies of Iodinated Glycerol (Organidin®) (CAS No. 5634-39-9) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of iodinated glycerol (Organidin®, a complex mixture prepared by the reaction of iodine with glycerol and found to contain 33% 3-iodo-1,2-propanediol as the major component) were conducted because of human exposure to iodinated glycerol as an expectorant and its possible relationship to the formation of alkyl iodides, e.g., methyl iodide, a suspected animal carcinogen. These studies were conducted by giving iodinated glycerol in water by gavage (5 days per week) to groups of F344/N rats and $B6C3F_1$ mice for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted with iodinated glycerol in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, Chinese hamster ovary (CHO) cells, and $B6C3F_1$ mice (in vivo bone marrow micronucleus test). Also, 3-iodo-1,2-propanediol was tested in *S. typhimurium* and $B6C3F_1$ mice (in vivo micronucleus assay).

Sixteen-Day and Thirteen-Week Studies: Sixteen-day studies were conducted by giving iodinated glycerol at doses up to 1,000 mg/kg to rats and up to 500 mg/kg to mice. All female rats and 4/5 male mice in the highest dose group died before the end of the studies; there were no dose-related effects on body weights of male or female rats or male mice at the end of the studies. The forestomach of 2/5 female mice that received 500 mg/kg was thickened and granular.

Thirteen-week studies were conducted by administering iodinated glycerol at doses up to 500 mg/kg to rats and mice. During these studies, 3/10 female rats and 1/10 female mice that received 500 mg/kg died. Final mean body weights of rats and mice that received 500 mg/kg were 4% lower than those of vehicle controls for males and 6%-7% lower for females.

Kidney tubular cell lesions, including cortical necrosis, regeneration, and calcification, were observed at increased incidences in the highest dose group of female rats. Lymphoid hyperplasia of the stomach was observed in dosed male and female rats. Kidney tubular cell regeneration was also observed in dosed female mice. Inflammation or abscesses of mild-to-moderate severity and hyperplasia, acanthosis, and/or hyperkeratosis of mild-to-moderate severity were observed in the forestomach of the highest dosed group of female mice.

Body Weight and Survival in the Two-Year Studies: Two-year studies were conducted by administering 0, 125, or 250 mg/kg iodinated glycerol in deionized water by gavage, 5 days per week for 103 weeks, to groups of 50 male F344/N rats and 50 male $B6C3F_1$ mice. Groups of 50 female F344/N rats and 50 female $B6C3F_1$ mice were administered iodinated glycerol on the same schedule at lower doses of 0, 62, or 125 mg/kg because of the increased severity of kidney and stomach lesions in the 13-week studies. Mean body weights of high dose male rats were 5%-10% lower than those of vehicle controls from week 43 to week 68 and 10%-13% lower from week 72 to the end of the studies. Mean body weights of low dose male rats and high dose female rats were 4%-9% lower than those of vehicle controls from week 88 to the end of the studies. The survival of the high dose group of male rats was considerably lower than that of the vehicle controls after week 86. No other significant differences in survival were observed between any groups of rats of either sex (male: vehicle control, 28/50; low dose, 20/50, high dose, 2/50; female: 31/50; 30/50; 27/50). Mean body weights of dosed and vehicle control male mice were similar. Mean body weights of high dose female mice were 6%-8% lower than those of vehicle controls from week 40 to week 64 and were 9%-13% lower thereafter. No significant differences in survival were observed between any groups of mice of either sex (male: 36/50; 40/50; 32/50; female: 40/50; 33/50; 38/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The incidence of mononuclear cell leukemia were increased in dosed male rats (vehicle control, 14/50; low dose, 29/50; high dose, 24/50).

Follicular cell carcinomas of the thyroid gland in male rats occurred at an increased incidence in low dose male rats (0/49; 5/49; 1/49). Reduced survival of high dose male rats may have been responsible for the decreased tumor incidence in this group relative to that in the low dose group. Follicular cell carcinomas were observed in one low dose and one high dose female rat. Follicular cell carcinomas of the thyroid gland have been observed in 3/293 water gavage vehicle control male F344/N rats and in 10/1,904 untreated control male F344/N rats.

Adenomas of the nasal cavity were observed in two high dose male rats. Adenomas of the nasal cavity have

not been observed in 300 water gavage vehicle control male F344/N rats or in 1,936 untreated control male F344/N rats.

Squamous metaplasia and focal atrophy of the salivary glands were observed at increased incidences in dosed rats (squamous metaplasia—male: 0/48; 47/50; 48/49; female: 1/49; 48/50; 49/50; focal atrophy—male: 1/48; 10/50; 30/49; female: 0/49; 4/50; 11/50).

In dosed female mice, adenomas of the anterior pituitary gland were increased (10/47; 15/45; 24/46). The incidences of adenomas of the harderian gland in dosed female mice were increased (6/50; 8/40; 13/50). A carcinoma of the harderian gland was observed in another high dose female mouse.

Dilatation of the thyroid gland follicle and follicular cell hyperplasia were observed at increased incidences in dosed mice (dilatation—male: 0/48; 28/50; 32/50; female: 4/48; 11/48; 10/48; hyperplasia—male: 3/48; 46/50; 34/50; female: 2/48; 25/48; 35/48). The incidences of follicular cell adenomas were 3/48, 6/50, and 0/50 for males and 2/48, 3/48, and 4/48 for females.

Hyperkeratosis and acanthosis of the forestomach were observed at increased incidences in high dose male mice (hyperkeratosis: 0/49; 0/49; 5/50; acanthosis: 0/49; 1/49; 5/50). Squamous cell papillomas were observed in female mice (1/49; 2/50; 5/49). The historical incidence of forestomach squamous cell neoplasms is 4/339 (1.2%) in water gavage vehicle control female $B6C3F_1$ mice and is 18/1,994 (0.9%) in untreated control female $B6C3F_1$ mice. Squamous cell neoplasms were not observed in male mice.

Genetic Toxicology: Treatment of the base-substitution mutant *S. typhimurium* strains TA100 and TA1535 with iodinated glycerol in a preincubational protocol with and without S9 resulted in a dose-related increase in the number of revertant colonies; no increase in revertants was observed with the frame-shift mutant strains TA98 or TA1537. 3-Iodo-1,2-propanediol was also mutagenic in TA100 with or without S9; it was not mutagenic in TA98. Iodinated glycerol increased the number of trifluorothymidine-resistant cells in mouse lymphoma L5178Y/TK$^{+/-}$ assay in the absence of exogenous metabolic activation; it was not tested with activation. Iodinated glycerol induced sister chromatid exchanges (SCEs) and chromosomal aberrations in CHO cells without S9; with S9, the frequency of SCEs was increased more than without S9 but no chromosomal aberrations were induced. No increase in micronucleated polychromatic erythrocytes was observed in the bone marrow of $B6C3F_1$ mice after injection with either iodinated glycerol or 3-iodo-1,2-propanediol.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* for male F344/N rats administered iodinated glycerol, as indicated by increased incidences of mononuclear cell leukemia and follicular cell carcinomas of the thyroid gland. Adenomas of the nasal cavity in two high dose male rats may have been related to the administration of iodinated glycerol. There was *no evidence of carcinogenic activity* for female F344/N rats administered 62 or 125 mg/kg iodinated glycerol by gavage for 103 weeks. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice administered 125 or 250 mg/kg iodinated glycerol by gavage for 103 weeks. There was *some evidence of carcinogenic activity* for female $B6C3F_1$ mice administered iodinated glycerol, as indicated by increased incidences of adenomas of the anterior pituitary gland and neoplasms of the harderian gland. Squamous cell papillomas of the forestomach may have been related to the administration of iodinated glycerol.

Significant nonneoplastic lesions considered related to exposure of iodinated glycerol were squamous metaplasia and focal atrophy of the salivary gland in male and female rats. Dilatation of the thyroid gland follicle and follicular cell hyperplasia were observed in male and female mice.

Synonyms or Trade Names: Organidin®; iodopropylidene glycerol

Report Date: March 1990

TR-341 Toxicology and Carcinogenesis Studies of Nitrofurantoin (CAS No. 67-20-9) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Nitrofurantoin was studied and evaluated because of its widespread use as a drug for treating urinary tract infections in humans, its structural relationship to known carcinogenic 5-nitrofuran compounds, and the lack of adequate studies to assess its carcinogenicity. Toxicology and carcinogenesis studies of nitrofurantoin were conducted by administering nitrofurantoin (greater than 99% pure) in feed to groups of F344/N rats and $B6C3F_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: None of the rats (at dietary concentrations up to 20,000 ppm) died before the end of the 14-day studies. Rats that received 5,000, 10,000, or 20,000 ppm lost weight. Four of five male and 4/5 female mice that received 10,000 ppm and 1/5 females that received 5,000 ppm nitrofurantoin died before the end of the studies. Mice that received 5,000 ppm and male mice that received 10,000 ppm lost weight.

In the 13-week studies, final mean body weights of rats that received 2,500, 5,000, or 10,000 ppm were 10%, 34%, or 47% lower than that of the controls for males and 15%, 31%, or 41% lower for females. Feed consumption by dosed and control rats was generally similar. Degeneration of the germinal epithelium of the seminiferous tubules of the testis was observed in male rats that received 2,500 to 10,000 ppm nitrofurantoin. Necrosis of the ovarian follicles was observed in 8/10 female rats that received 10,000 ppm, in 3/10 females that received 5,000 ppm, and in 1/10 that received 2,500 ppm.

For mice, final mean body weights of the 5,000-ppm groups were 13% lower than that of the controls for males and 15% lower for females. Two of 10 male mice that

received 5,000 ppm and 1/10 males that received 300 ppm died before the end of the 13-week studies. Estimated feed consumption was similar for dosed and control groups. Degeneration of the germinal epithelium of the testis was observed in males that received 1,300 to 5,000 ppm; necrosis of the ovarian follicles was observed in females that received 5,000 ppm but not in the lower dose groups. Necrosis of the renal tubular epithelium was observed in 2/9 males that received 5,000 ppm.

Based on these results, 2-year studies of nitrofurantoin were conducted by feeding diets containing 0, 1,300, or 2,500 ppm nitrofurantoin to groups of 50 male F344/N rats and to groups of 50 male and female $B6C3F_1$ mice for 103 weeks. Groups of 50 female F344/N rats were fed diets containing 0, 600, or 1,300 ppm nitrofurantoin on the same schedule.

Body Weight and Survival in the Two-Year Studies: Mean body weight and average daily feed consumption of dosed male and female rats were similar to those of the controls throughout the studies. The average amount of nitrofurantoin consumed per day was estimated to be 60 and 110 mg/kg for low and high dose male rats and 30 and 60 mg/kg for low and high dose female rats. No significant differences in the number of rats surviving to the end of the studies were observed between any groups of rats of either sex (male: control, 24/50; low dose, 27/50; high dose, 26/50; female: 25/50; 26/50; 31/50).

Mean body weights of high dose male and female mice were up to 12% lower than those of the controls throughout most of the studies. The average daily feed consumption by dosed mice ranged from 93% to 100% that by controls. The average amount of nitrofurantoin consumed per day was estimated to be 280-300 mg/kg and 570-580 mg/kg for low and high dose mice. The survival of the control group of female mice was lower than that of the dosed groups (control, 19/50; low dose, 37/50; high dose, 37/50). The decrease in survival was most likely related to the increase in microbial infection in the reproductive tract observed in the controls. Groups of male mice had similar survival (28/50; 29/50; 34/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Organs showing toxicity from nitrofurantoin exposure identified in the short-term studies were the testis in male rats and mice, the ovary in female rats and mice, and the kidney in male mice. Lesions observed in the 2- year studies were in the testis in male rats and mice, ovary in female mice, and kidney in male rats.

Chronic nephropathy was observed in nearly all rats, but the severity of the lesions was judged to be greater in dosed male rats. Hyperplasia of the transitional cell epithelium (control, 0/50; low dose, 5/50; high dose, 2/50) and hydronephrosis of the renal pelvis (0/50; 5/50; 2/50) were also observed in dosed male rats. In the standard single sections of the left and right kidney from each rat, tubular cell adenomas were observed in one low dose and two high dose males; a tubular cell carcinoma was observed in another high dose male. Because the number of renal tubular cell neoplasms identified by standard procedures in the dosed male rats was low, additional step-sections of the kidney were evaluated. The incidences of tubular cell adenomas derived from the step-sections and original sections (combined) were significantly increased in dosed male rats (adenomas: 3/50; 11/50; 19/50); tubular cell carcinomas occurred in two high dose males only.

Lesions considered to be associated with the nephropathy and nitrofurantoin exposure were observed in male rats and included hyperplasia of the parathyroid glands (3/49; 18/47; 23/49), fibrous osteodystrophy of the bone (0/50; 5/50; 5/50), and mineralization of the glandular stomach (1/49; 8/50; 14/50).

Atypical cells of the epididymis (0/50; 0/50; 12/50) and degeneration of the testis (0/50; 0/50; 36/50) were observed in high dose male rats. Fibrinoid necrosis of arterioles (1/50; 8/50; 15/50) and perivascular infiltration of mononuclear cells (3/50; 9/50; 19/50) were also observed in the testis of male rats. Interstitial cell adenomas of the testis occurred with a negative trend (47/50; 45/50; 21/50), and no adenomas or carcinomas of the preputial gland were seen in high dose male rats (12/48; 11/50; 0/47). The incidence of clitoral gland neoplasms was increased in low dose female rats (5/44; 10/38; 4/42).

Osteosarcomas were observed in the bone of one low dose and two high dose male rats. The historical incidence of osteosarcomas in untreated male F344/N rats is 8/1,937 (0.4%). The incidences of subcutaneous tissue neoplasms in dosed male rats were greater than that in the controls (1/50; 7/50; 5/50).

No neoplastic lesions in dosed female rats or male mice were considered to be compound related at the doses of nitrofurantoin administered.

For female mice, ovarian atrophy was observed in 48/50 low dose and 49/50 high dose mice but not in controls. Tubular cell adenomas of the ovary (0/50; 0/50; 5/50), benign mixed tumors (tubular and stromal) (0/50; 0/50; 4/50), and granulosa cell tumors (0/50; 3/50; 2/50)) were observed in dosed female mice. One granulosa cell tumor in the high dose group was malignant. Ovarian abscesses (18/50) and suppurative inflammation of the uterus (11/50) were observed in control female mice but not in dosed female mice and are believed to be related to indigenous microbial infections and most likely were the cause of early deaths in this group. Adenocarcinomas of the uterus were seen in one low dose and in one high dose mouse.

Testicular aspermatogenesis (1/49; 1/49; 16/50), degeneration of the germinal epithelium (0/49; 3/49; 23/50), and atypical cells (0/50; 0/49; 26/50) and depletion (1/50; 1/49; 15/50) of the epididymis were observed at increased incidences in high dose male mice.

Spindle cell hyperplasia of the adrenal cortex was observed in dosed female mice (3/50; 41/50; 45/50). A spindle cell adenoma (adrenal capsule adenoma) was seen in one low dose female mouse, and a spindle cell carcinoma (adrenal capsule carcinoma) was seen in one low dose male mouse.

Mineralization of the renal medulla (male: 0/50; 0/50; 17/50; female: 0/50; 0/50; 7/50) and dilatation of the renal tubules (male: 0/50; 0/50; 14/50) were observed in high dose mice.

Hepatocellular neoplasms (adenomas or carcinomas, combined) were observed at an increased incidence in high dose female mice (2/50; 2/50; 8/50). An Ito cell tumor of the liver was observed in one low dose and one high dose female mouse. Malignant lymphomas occurred in female mice (12/50; 19/50; 24/50).

Genetic Toxicology: Nitrofurantoin was mutagenic in *Salmonella typhimurium* strains TA98 and TA100, with and without metabolic activation, but was not mutagenic for strains TA1535 or TA1537. Nitrofurantoin induced forward mutations at the $TK^{+/-}$ locus of L5178Y mouse lymphoma cells in the absence of metabolic activation (it was not tested with activation). Nitrofurantoin induced increased numbers of sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells with and without metabolic activation. Results of the sex-linked recessive lethal assay in Drosophila were negative after administration of nitrofurantoin by feeding or by injection.

Conclusions: Under the conditions of these 2-year feed studies, there was *some evidence of carcinogenic activity* of nitrofurantoin for male F344/N rats as shown by increased incidences of uncommon kidney tubular cell neoplasms. Uncommon osteosarcomas of the bone and neoplasms of the subcutaneous tissue were observed in dosed male rats. Incidences of interstitial cell adenomas of the testis and neoplasms of the preputial gland were decreased in the 2,500-ppm group of male rats. There was *no evidence of carcinogenic activity* of nitrofurantoin for female F344/N rats fed diets containing 600 ppm or 1,300 ppm for 2 years. Female rats may have been able to tolerate higher doses. There was *no evidence of carcinogenic activity* of nitrofurantoin for male $B6C3F_1$ mice fed diets containing 1,300 ppm or 2,500 ppm for 2 years. There was *clear evidence of carcinogenic activity* of nitrofurantoin for female $B6C3F_1$ mice as shown by increased incidences of tubular adenomas, benign mixed tumors, and granulosa cell tumors of the ovary.

Nonneoplastic lesions considered related to nitrofurantoin exposure were chronic nephropathy and associated lesions (hyperplasia of the parathyroid gland, fibrous osteodystrophy of the bone, and mineralization of the glandular stomach) in male rats and testicular degeneration in male rats and mice. Ovarian atrophy and hyperplasia of the adrenal cortex spindle cells were observed in dosed female mice.

Synonyms: 1-(((5-nitro-2-furanyl)methylene)amino-2,4-imidazolidinedione); 1-(5-nitro-2-furfurylideneamino)-hydantoin; *N*-(5-nitro-2-furfurlidene)-1-aminohydantoin; 1-((5-nitrofurfurylidene)amino)hydantoin

Trade Names: Benkfuran; Benkfurin; Chemiofuran; Cyantin; Dantafur; Furadantin; Furadantine; Furadantoin; Furadonin; Furadonine; Furantoin; Furatoin; Furobactina; Ituran; Macrodantin; Nifurantin; NSC 2107; N-Toin; Orafuran; Parafuran; Urizept; USAF EA-2; Welfurin; Zoofurin

Report Date: September 1989

TR-342 Toxicology and Carcinogenesis Studies of Dichlorvos (CAS No. 62-73-7) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of dichlorvos (99% pure), a contact and stomach poison for control of insects and parasites, were conducted by administering dichlorvos in corn oil by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 13 weeks or 2 years. Previous feed studies were done by the National Cancer Institute using Osborne-Mendel rats and $B6C3F_1$ mice.

Thirteen-Week Studies: Thirteen-week studies with groups of 10 rats of each sex were conducted at doses of 0, 2, 4, 8, 16, 32, or 64 mg/kg dichlorvos in corn oil. All rats that received 32 or 64 mg/kg dichlorvos and 4/10 females that received 16 mg/kg died before the end of the studies. Final mean body weights of dosed and vehicle control rats were similar. Thirteen-week studies with groups of 10 mice of each sex were conducted at doses of 0, 5, 10, 20, 40, 80, or 160 mg/kg. All 10 male mice and 9/10 female mice that received 160 mg/kg and 5/10 male mice that received 80 mg/kg dichlorvos died before the end of the studies. Final mean body weights of dosed and vehicle control mice were similar. No compound-related gross or microscopic pathologic effects were observed in rats or mice.

Two-year studies of dichlorvos were conducted by administering 0, 4, or 8 mg/kg dichlorvos, 5 days per week for 103 weeks, to groups of 50 F344/N rats of each sex. Groups of 50 male $B6C3F_1$ mice were administered 0, 10, or 20 mg/kg dichlorvos on the same schedule, and groups of 50 $B6C3F_1$ female mice were administered 0, 20, or 40 mg/kg dichlorvos.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed and vehicle control rats and mice were similar. No significant differences in survival were observed between any groups of rats or mice of either sex (rats—male: vehicle control, 31/50; low dose, 25/50; high dose, 24/50; female: 31/50; 26/50; 26/50; mice—male: 35/50; 27/50; 29/50; female: 26/50; 29/50; 34/50).

Neoplastic Effects in the Two-Year Studies: Adenomas of the exocrine pancreas occurred at greater incidences in dosed rats than in vehicle controls (male: vehicle control, 25/50; low dose, 30/49; high dose, 33/50; female: 2/50; 3/47; 6/50). Mononuclear cell leukemia in both dosed groups of male rats occurred more frequently than in vehicle controls (11/50; 20/50; 21/50). Mammary gland fibroadenomas and fibroadenomas or adenomas (combined) in dosed female rats occurred at increased incidences relative to the vehicle controls (9/50; 19/50; 17/50). Multiple fibroadenomas occurred in dosed female rats but not in vehicle controls (0/50; 6/50; 3/50); carcinomas occurred in two vehicle control and two low dose female rats.

In mice, incidences of squamous cell papillomas of the forestomach were increased in the high dose groups compared with those in the vehicle controls (male: 1/50;

1/50; 5/50; female: 5/49; 6/49; 18/50). Two high dose female mice developed forestomach squamous cell carcinomas.

Genetic Toxicology: Dichlorvos was mutagenic in *Salmonella typhimurium* strain TA100 with and without metabolic activation but was not mutagenic in strain TA98. Dichlorvos was mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay without metabolic activation. Dichlorvos induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells in the absence and presence of metabolic activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of dichlorvos for male F344/N rats, as shown by increased incidences of adenomas of the exocrine pancreas and mononuclear cell leukemia. There was *equivocal evidence of carcinogenic activity* of dichlorvos for female F344/N rats, as shown by increased incidences of adenomas of the exocrine pancreas and mammary gland fibroadenomas. There was *some evidence of carcinogenic activity* of dichlorvos for male B6C3F$_1$ mice, as shown by increased incidences of forestomach squamous cell papillomas. There was *clear evidence of carcinogenic activity* of dichlorvos for female B6C3F$_1$ mice, as shown by increased incidences of forestomach squamous cell papillomas.

Synonyms: 2,2-dichloroethenyl dimethyl phosphate; 2,2-dichlorovinyl dimethyl phosphate; *O,O*-dimethyl-*O*-(2,2-dichlorovinyl)phosphate; DDVP

Trade Names: BAY-19149; DDVF; ENT-20738; OMS-14; SD 1750; Canogard®; Crossman's Fly-Cake®; Dedevap®; De-Pester Insect Strip®; Estrosol®; Herkol®; Kill-fly Resin Strip®; Lethalaire®; Mafu®; Misect®; Nogos®; Nuvan®; No-Pest Strip®; Oko®; Phoracide®; Phosvit®; Vapona®; Vaponicide®; Vaporette Bar®

Anthelmintics: Atgard®; Dichlorman®; Equigard®; Task®

Report Date: September 1989

Note: Dichlorvos (technical grade) was previously tested in Osborne-Mendel rats and B6C3F$_1$ mice administered in feed (See TR-10, reported 1979).

TR-343 Toxicology and Carcinogenesis Studies of Benzyl Alcohol (CAS No. 100-51-6) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of technical-grade benzyl alcohol (99% pure), a textile dye additive, solvent, and food flavoring agent, were conducted by administering the chemical by gavage in corn oil vehicle to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days, 13 weeks, or 2 years.

Short-Term Studies: In 16-day studies, all five male and five female rats and mice dosed with 2,000 mg/kg benzyl alcohol died. Two of five male and 3/5 female rats and 1/5 male and 2/5 female mice dosed with 1,000 mg/kg died. Rats and mice of each sex in the two highest dose groups were lethargic after dosing. Other toxic responses to benzyl alcohol in these dose groups included blood around the mouth and nose, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tracts of rats and blood in the urinary bladder of mice. Animals administered lower doses of benzyl alcohol (125, 250, or 500 mg/kg) had no compound-related histologic lesions.

Doses selected for the 13-week studies were 0, 50, 100, 200, 400, and 800 mg/kg for rats and mice. Eight of 10 male rats dosed with 800 mg/kg died during weeks 7 and 8; four of these deaths were described as gavage related. Rats dosed with 800 mg/kg exhibited clinical signs indicative of neurotoxicity including staggering, respiratory difficulty, and lethargy. Hemorrhages occurred around the mouth and nose, and there were histologic lesions in the brain, thymus, skeletal muscle, and kidney. In mice, deaths were scattered among all dose levels, but none occurred in vehicle controls. Four male and six female mice died after being dosed; all deaths but one were described as gavage related. Staggering after dosing also occurred during the first 2 weeks of the studies in mice dosed with 800 mg/kg. Some of the deaths in the rats and mice may have been caused by a combination of the gavage procedure and chemical toxicity, since there was evidence that benzyl alcohol induced neurotoxic effects. There were reductions in relative weight gain in male rats dosed with 800 mg/kg benzyl alcohol, in female rats dosed with 200 mg/kg or more, in male mice dosed with 400 or 800 mg/kg, and in female mice dosed with 200 mg/kg or more. No notable changes in body weight gain or compound-related histopathologic lesions were observed in rats or mice from the lower dose groups. Based on mortality, reduction in relative body weight gain, and the histopathologic lesions, doses selected for 2-year studies in rats were 0, 200, and 400 mg/kg. Doses selected for 2-year studies in mice were 0, 100, and 200 mg/kg, based on mortality and depression in relative body weight gain.

Body Weight and Survival in the Two-Year Studies: Fifty animals of each species and sex were administered benzyl alcohol in corn oil by gavage 5 days per week for 103 weeks. Administration of benzyl alcohol did not affect survival in male rats (final survival rates: vehicle control, 28/50; low dose, 27/50; high dose, 24/50) but reduced survival of dosed female rats by half (36/50; 18/50; 17/50). Many of the early deaths were considered related to the gavage procedure. Survival in mice was not affected by benzyl alcohol administration (male: 34/50; 33/50; 35/50; female: 26/50; 32/50; 36/50). No effect of benzyl alcohol on body weight gain in rats or mice was observed. In the third month of the studies, clinical signs of sialodacryoadenitis virus infection were observed in rats. A positive serologic reaction for rat coronavirus was observed in sentinel animals at 6 months and again at 18 months.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: No apparent compound-related nonneoplastic responses were observed. Dose-related negative trends

in the incidences of anterior pituitary gland neoplasms were seen in female rats (vehicle control, 29/50; low dose, 17/47; high dose, 9/49) and of Harderian gland adenomas in male mice (8/50; 3/50; 2/50). Adenomas of the adrenal cortex occurred at an increased incidence in high dose male mice (0/48; 0/44; 3/48), but this slight increase was not considered to be related to chemical exposure.

Genetic Toxicology: Benzyl alcohol was not mutagenic when tested by the preincubational protocol in the presence or absence of exogenous metabolic activation in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537. In the mouse L5178Y/TK $^{+/-}$ lymphoma assay, benzyl alcohol induced an increase in trifluorothymidine (Tft)-resistant cells in the absence, but not in the presence, of S9; the effect was associated with toxicity. In cytogenetic assays with Chinese hamster ovary (CHO) cells, treatment with benzyl alcohol produced an increase in sister chromatid exchanges (SCEs) which was judged to be equivocal both with and without S9; a significant increase in chromosomal aberrations was observed after exposure to benzyl alcohol in the presence, but not the absence, of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of benzyl alcohol have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of benzyl alcohol for male or female F344/N rats dosed with 200 or 400 mg/kg. Survival in both dose groups of female rats was 50% that of vehicle controls, primarily due to an increased number of gavage-related deaths. There was *no evidence of carcinogenic activity* of benzyl alcohol for male or female B6C3F$_1$ mice dosed with 100 or 200 mg/kg for 2 years.

Synonyms: benzenemethanol; phenylcarbinol; phenylmethanol; α-hydroxytoluene; benzenecarbinol; phenolcarbinol; α-toluenol

Report Date: June 1989

TR-344 Toxicology and Carcinogenesis Studies of Tetracycline Hydrochloride (CAS No. 64-75-5) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Tetracycline hydrochloride is a broad-spectrum antibiotic used for its bactericidal action in human and veterinary medicine. Toxicology and carcinogenesis studies of tetracycline hydrochloride (USP grade, 91% pure) were conducted by feeding diets containing tetracycline hydrochloride to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: The same dietary concentrations were used for the 14-day and 13-week studies (0, 3,125, 6,250, 12,500, 25,000 and 50,000 ppm tetracycline hydrochloride). In the 14-day studies, none of the rats or mice died. The final mean body weight of male rats that received 50,000 ppm was 24% lower than that of the controls. The final mean body weight of mice that received 50,000 ppm in the diet was 18% lower than that of the controls for males and 15% lower for females. No compound-related effects were observed in rats or mice at necropsy.

During the 13-week studies, none of the rats or mice died. The final mean body weight of male rats that received 50,000 ppm was 18% lower than that of the controls. Compound-related effects included cytoplasmic vacuolization in the liver of male rats at 25,000 and 50,000 ppm. Bone tetracycline concentrations in rats and mice increased with increasing dose of tetracycline hydrochloride. The final mean body weight of mice that received 50,000 ppm was 16% lower than that of the controls for males and 6% lower for females. Estimated feed consumption by dosed rat and mouse groups was similar to that of the controls. No compound-related gross or microscopic pathologic effects were observed in mice.

Based on these results, 2-year studies of tetracycline hydrochloride were conducted by feeding diets containing 0, 12,500, or 25,000 ppm tetracycline hydrochloride to groups of 50 rats and 50 mice of each sex for 103 weeks.

Body Weight, Survival, and Feed Consumption in the Two-Year Studies: Mean body weights of dosed and control male and female rats were similar throughout most of the studies. The survival of both the low and high dose female groups was greater than that of the controls. No significant differences in survival were observed between any groups of male rats (male: control, 27/50; low dose, 24/50; high dose, 31/50; female: 27/50; 39/50; 38/50). Mean body weights of dosed mice were markedly (more than 10%) lower than those of the controls throughout most of the studies. The survival rates of the dosed groups of male mice were greater than that of the control group. No significant differences in survival were observed between any groups of female mice (male: 31/50; 43/50; 43/50; female: 37/50; 35/50; 38/50). Feed consumption was similar by dosed and control rats and mice of either sex throughout the studies.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Basophilic cytoplasmic change and clear cell change were positively correlated with tetracycline hydrochloride administration in male rats. Otherwise, no significant increases in neoplastic or nonneoplastic lesions in rats or mice of either sex were considered related to tetracycline hydrochloride administration.

The incidence of adenomas or carcinomas (combined) of the pancreatic islets in low dose male rats was greater than that in the controls (control, 0/49; low dose, 5/49; high dose, 0/49). This marginal effect in the low dose group was not considered to be chemically related. The historical control rate of pancreatic islet cell neoplasms from previous studies at this laboratory is 6% (9/148).

Decreased incidences and severity of chronic nephropathy in male rats were associated with tetracycline hydrochloride administration (48/50; 35/50; 36/50). Female mice administered tetracycline hydro-

chloride in feed did not develop hepatocellular adenomas or carcinomas (combined incidence: 10/49; 0/48; 0/50). The historical control rate for hepatocellular adenomas or carcinomas (combined) from previous studies at this laboratory is 18/149 (12%). Other decreases in tumor incidence involving several tissues were considered to be of marginal biologic significance.

Genetic Toxicology: Tetracycline hydrochloride was not mutagenic in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, or TA1537) when tested in a preincubation protocol in the presence or absence of exogenous metabolic activation. Tetracycline hydrochloride was negative in the mouse lymphoma L5178Y/TK$^{+/-}$ assay with or without induced rat liver S9 but gave a marginally positive response when tested in the presence of noninduced S9. In cytogenetic assays with Chinese hamster ovary (CHO) cells, treatment with tetracycline hydrochloride, both with and without S9, did not induce chromosomal aberrations or sister chromatid exchanges (SCEs). Tetracycline hydrochloride did not induce sex-linked recessive lethal mutations when administered by feeding or injection to adult male Drosophila.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* of tetracycline hydrochloride for male or female F344/N rats and B6C3F$_1$ mice fed diets containing 12,500 or 25,000 ppm. Tetracycline hydrochloride-dosed female rats and male mice had greater survival rates than the respective controls during these studies. Dosed mice had lower body weight than controls, and dosed female mice had no hepatocellular adenomas or carcinomas.

Trade Names for Tetracycline or Tetracycline hydrochloride: Achromycin; Amycin; Bristacycline; Cyclopar; Dumocyclin; Neocyclin B; Panmycin; Polycycline; Robitet; Ro-cycline; Steclin; Sumycin; Topicycline; Unimycin

Report Date: August 1989

TR-345 Toxicology and Carcinogenesis Studies of Roxarsone (CAS No. 121-19-7) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Roxarsone is a veterinary drug used as a growth promoter and as an anticoccidial agent and for treatment of swine dysentery. Toxicology and carcinogenesis studies were conducted by administering roxarsone (greater than 99.4% pure) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, the diets fed to rats contained 0 or 100-1,600 ppm roxarsone, and those fed to mice contained 0 or 60-1,000 ppm. Deaths occurred in rats and mice that received the highest doses. Rats that received 800 or 1,600 ppm lost weight. Male mice that received 1,000 ppm and female mice that received 500 ppm lost weight.

In the first 13-week studies, roxarsone was fed to rats and mice at dietary concentrations of 0 or 50-800 ppm. Decreases (more than 10%) in final mean body weights of dosed rats relative to those of controls were observed for males that received 200, 400, or 800 ppm and for females that received 400 or 800 ppm. Deaths occurred in groups that received 800 ppm. Clinical signs of toxicity (trembling, ataxia, and pale skin) were seen primarily in rats that received 800 ppm. Kidney lesions were observed in rats that received 800 ppm. These lesions were characterized by tubular necrosis and mineralization in the rats that died during the studies and by tubular dilatation and casts, interstitial inflammation, and tubular epithelial cell regeneration in the rats that lived to the end of the studies.

Additional 13-week studies were conducted in rats at dietary concentrations of 0, 100, or 400 ppm to demonstrate the absorption of roxarsone from the gastrointestinal tract; to determine its distribution in liver, kidney, and blood; and to study its effects on various hematologic and clinical chemical values. No deaths occurred. Renal lesions of minimal severity observed in male rats that received 400 ppm were characterized by tubular epithelial cell degeneration and regeneration, tubular casts, and mineralization. Arsenic levels in urine, blood, kidney, and liver of dosed rats increased (140%-300%) with time on study and were proportional to the dietary concentrations of roxarsone. No compound-related hematologic or clinical chemical effects were observed in rats.

In the first 13-week studies, final mean body weights of mice that received 800 ppm were 11%-18% lower than those of controls. Deaths occurred in males and females receiving 400 and 800 ppm. No compound-related gross or histopathologic lesions were observed.

In the second 13-week studies in mice, no compound-related hematologic or clinical chemical effects were observed. At the end of the studies, arsenic concentrations in dosed mice ranged from 0.45 to 0.99 μg/g of liver and from 0.85 to 2.98 μg/g of kidney. No arsenic was detected in the liver or kidney of control mice.

Because of kidney lesions, lower body weight gain, and increased mortality in rats and lower body weight gain and increased mortality in mice in the short-term studies, dietary concentrations of roxarsone selected for the 2-year studies were 0, 50, or 100 ppm for rats and 0, 100, or 200 ppm for mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were generally within 5% of those of controls. No significant differences in survival were observed between any groups of rats of either sex, although survival in males was lower than usual (final survival—male: control, 24/50; low dose, 18/50; high dose, 18/50; female: 27/50; 35/50; 32/50). The average feed consumption by high dose rats was 95% that of controls for males and 88% for females. The average amount of roxarsone consumed per day was approximately 2 mg/kg for low dose rats and 4 mg/kg for high dose rats. Mean body weights of high dose male mice were generally 5%-8% higher than those of the

controls, whereas those of female mice were generally 6%-15% lower than those of the controls. The survival of the control group of male mice was lower than that of the low dose group after month 22; survival for females was low (final survival—male: 27/50; 40/50; 33/50; female: 14/50; 18/50; 17/50). The low survival in females was due in part to utero-ovarian infection, with more than 50% of the animals in each dose group having suppurative inflammation at this site. The average daily feed consumption by dosed mice was 105%-110% that by the controls. The average amount of roxarsone consumed per day was approximately 21 or 43 mg/kg for low dose or high dose male mice and 27 or 54 mg/kg for low dose or high dose female mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Although the incidence of adenomas of the exocrine pancreas in high dose male rats was not statistically greater than that in the controls (control, 1/50; low dose, 1/50; high dose, 5/50), it was greater than that seen in any historical control group of male F344/N rats. The historical rate is 1/437 (0.2%) for the study laboratory and 5/1,871 (0.3%) throughout the Program. The incidences of hyperplasia were 2/50; 0/50; 3/50. No hyperplasia or adenomas were observed in the exocrine pancreas of female rats.

Clitoral gland adenomas in female rats occurred with a marginally positive trend (1/44; 3/47; 6/48; $P = 0.049$). One carcinoma was also observed in each of the groups. The incidences of adenomas or of adenomas or carcinomas (combined) in the dosed groups were not significantly different from those in the controls. This marginal effect was not considered to be related to roxarsone administration.

No chemical-related increases in neoplastic or nonneoplastic lesions occurred in male or female mice. Lymphomas in female mice occurred with a negative trend; the incidences in the dosed groups were lower than that in the controls (13/50; 2/50; 3/50; $P \leq 0.01$).

Genetic Toxicology: Roxarsone was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation. Roxarsone induced trifluorothymidine (Tft) resistance in mouse lymphoma L5178Y cells in the absence of metabolic activation; it was not tested with activation. Exposure of adult male *Drosophila melanogaster* to roxarsone by injection or by feeding did not cause an increase in sex—linked recessive lethal mutations.

Audit: The data, documents, and pathology materials from the 2-year studies of roxarsone have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of roxarsone for male F344/N rats, as indicated by a marginally increased incidence of adenomas of the exocrine pancreas. There was *no evidence of carcinogenic activity* for female F344/N rats fed diets containing 50 or 100 ppm roxarsone for 2 years. There was *no evidence of carcinogenic activity* for male or female $B6C3F_1$ mice fed diets containing 100 or 200 ppm roxarsone for 2 years.

Synonyms: 4-hydroxy-3-nitrophenylarsonic acid; 4-hydroxy-3-nitrobenzenearsonic acid; 2-nitro-1-hydroxybenzene-4-arsonic acid; nitrophenolarsonic acid; 3-nitro-4-hydroxybenzenearsonic acid; 3-nitro-4-hydroxyphenylarsonic acid

Trade Names: Ristat; Ren-O-sal; 3-nitro; 3-nitro-10; 3-nitro-20; 3-nitro-50; 3-nitro-80

Report Date: March 1989

TR-346 Toxicology and Carcinogenesis Studies of Chloroethane (Ethyl Chloride) (CAS No. 75-00-3) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

Toxicology and carcinogenesis studies of chloroethane (99.5% pure), an alkylating agent and chemical intermediate, as well as a topical and inhalation anesthetic, were conducted by exposing groups of F344/N rats and $B6C3F_1$ mice of each sex to chloroethane by whole-body inhalation once for 4 hours or for 6 hours per day, 5 days per week for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*.

Single-Exposure, Fourteen-Day, and Thirteen-Week Studies: In the single-exposure and 14-day inhalation studies, all rats and mice exposed to 19,000 ppm chloroethane survived. The animals were not exposed at lower concentrations. No clinical signs of toxicity were seen. In the 14-day studies, final mean body weights of exposed male rats and exposed mice were higher than those of controls. Mean body weights of exposed and control female rats were similar.

In the 13-week studies, rats and mice were exposed to 0, 2,500, 5,000, 10,000, or 19,000 ppm chloroethane. No compound-related deaths occurred in rats or mice. The final mean body weight of rats exposed to 19,000 ppm was 8% lower than that of controls for males and 4% lower for females. Final mean body weights of exposed mice were generally higher than those of controls. No compound-related clinical signs or gross or microscopic pathologic effects were seen in rats or mice. The liver weight to body weight ratios for male rats and female mice exposed to 19,000 ppm were greater than those for controls. Although no chemically related toxic effects were observed in the short-term studies, concerns about potential flammability and explosion led to the selection of 0 and 15,000 ppm as the exposure concentrations for rats and mice for the 2-year studies.

Body Weight and Survival in the Two-Year Studies: Mean body weights of exposed male rats were 4%-8% lower than those of controls after week 33. Mean body weights of exposed female rats were generally 5%-13% lower than those of controls throughout the study. Although survival of male rats and exposed female rats was low at the end of the studies (male: control, 16/50;

exposed, 8/50; female: 31/50; 22/50), no statistically significant differences in survival were observed between exposed and control groups of either sex. Survival at week 90 for male rats was 37/50 (control) and 31/50 (exposed) and for females, 43/50 (control) and 33/50 (exposed). The high incidence of mononuclear cell leukemia may have contributed to the high mortality.

Mean body weights of exposed male mice were up to 13% higher than those of controls throughout the study. Mean body weights of exposed and control female mice were generally similar throughout the study. The survival of the exposed groups of both male (after day 330) and female (after day 574) mice was significantly lower than that of controls (final survival—male: 28/50; 11/50; female: 32/50; 2/50). The majority of exposed female mice died as a result of uterine carcinomas. Male mice, and particularly exposed mice, died early as a result of an ascending urinary tract infection.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Malignant astrocytomas of the brain were seen in three exposed female rats, and gliosis was observed in a fourth. The historical incidence of glial cell neoplasms in untreated control female F344/N rats is 23/1,969. The highest incidence observed in an untreated control group is 3/50.

Trichoepitheliomas (1/50), sebaceous gland adenomas (1/50), basal cell carcinomas (3/50), and squamous cell carcinomas (2/50) of the skin were observed only in exposed male rats. Keratoacanthomas occurred in four control and two exposed male rats.

Exposure of female mice to chloroethane caused a high incidence of uterine carcinomas of endometrial origin (control, 0/49; exposed, 43/50). One control female did have a uterine carcinoma, although it was not of endometrial origin. The tumors observed in 34 exposed females were highly malignant, invading the uterine myometrium and metastasizing to a wide variety of organs, primarily lung (23), ovary (22), lymph nodes (18), kidney (8), adrenal gland (8), pancreas (7), mesentery (7), urinary bladder (7), spleen (5), and heart (4), and to a lesser extent, colon, stomach, gallbladder, small intestine, ureter, and liver.

Two marginally increased incidences of other neoplasms were observed in exposed male and female mice. The incidence of hepatocellular carcinomas in exposed female mice was greater than that in controls (3/49; 7/48). One other exposed female had a hepatocellular adenoma. The incidence of alveolar/bronchiolar neoplasms of the lung in exposed male mice was greater than that in controls (adenomas or carcinomas, combined: 5/50; 10/48).

Genetic Toxicology: Chloroethane, tested within the closed environment of a desiccator, was mutagenic with and without exogenous metabolic activation in *S. typhimurium* strain TA1535; in strain TA100, a positive response was observed only with activation. No mutagenic activity was observed in *S. typhimurium* strain TA98 with or without metabolic activation.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *equivocal evidence of carcinogenic activity* of chloroethane for male F344/N rats, as indicated by benign and malignant epithelial neoplasms of the skin. For female F344/N rats, there was *equivocal evidence of carcinogenic activity*, as indicated by three uncommon malignant astrocytomas of the brain in the exposed group. The study of male $B6C3F_1$ mice was considered to be an *inadequate study of carcinogenicity* because of reduced survival in the exposed group. However, there was an increased incidence of alveolar/bronchiolar neoplasms of the lung. There was *clear evidence of carcinogenic activity* for female $B6C3F_1$ mice, as indicated by carcinomas of the uterus. A marginally increased incidence of hepatocellular neoplasms was observed in the exposed group.

Synonyms: monochloroethane; chloroethyl; ether hydrochloric; ether muriatic; aethylis; aethylis chloridum; ether chloridum; ether chloratus

Trade Names: Kelene; Chelen; Anodynon; Chloryl Anesthetic; Narcotile

Report Date: October 1989

TR-347 Toxicology and Carcinogenesis Studies of *d*-Limonene (CAS No. 5989-27-5) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of *d*-limonene, a naturally occurring monoterpene found in many volatile oils, especially in citrus oils, were conducted because of its widespread use as a flavor and fragrance additive for food and household cleaning products and its increasing use as an industrial solvent. The *d*-limonene used in these studies was more than 99% pure and was administered in corn oil by gavage. Short-term studies were conducted in F344/N rats and $B6C3F_1$ mice to identify toxic effects and affected sites and to help establish doses for the 2-year studies. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y cells, and Chinese hamster ovary (CHO) cells.

The doses selected for the 16-day studies ranged from 413 to 6,600 mg/kg for both rats and mice; deaths and reduction in body weight gain occurred at the two highest doses. No compound-related clinical signs or histopathologic lesions were observed in any of the surviving dose groups.

In the 13-week studies, doses of *d*-limonene ranged from 150 to 2,400 mg/kg for rats and from 125 to 2,000 mg/kg for mice. Deaths occurred in the high dose group of each species and sex. Greater than 10% reductions in body weight gain were observed in the two highest dose groups of male rats and male mice and the high dose female rats. Rough hair coats and decreased activity were observed at the two highest doses in both rats and mice. There were no chemical-related histopathologic lesions in female rats or in mice of either sex. A compound-related increased severity of nephropathy was observed in the kidney of male rats. This lesion was

characterized by degeneration of epithelial cells in the convoluted tubules, granular casts in the outer stripe of the outer medulla, and epithelial regeneration. These lesions have been described as reasonably characteristic of the hyaline droplet nephropathy that is associated with an accumulation of liver-generated $\alpha_2\mu$-globulin in the cytoplasm of tubular epithelial cells.

Two-year studies of *d*-limonene were conducted by administering 0, 75, or 150 mg/kg *d*-limonene in corn oil by gavage to groups of 50 F344/N male rats, 5 days per week for 103 weeks; groups of 50 female F344/N rats were administered 0, 300, or 600 mg/kg. These doses were selected based on compound-related, potentially life-threatening kidney lesions observed in males at 300 mg/kg and higher and on the large number of deaths of female rats at 2,400 mg/kg. Groups of 50 male $B6C3F_1$ mice were administered 0, 250, or 500 mg/kg according to the same schedule; groups of 50 female $B6C3F_1$ mice were administered 0, 500, or 1,000 mg/kg. These doses were selected based on the deaths observed for both male and female mice at 2,000 mg/kg during the 13-week studies and the body weight depression in male mice at 1,000 mg/kg and higher.

Mean body weights of rats dosed with *d*-limonene were similar to those of vehicle controls throughout the studies. Survival of the high dose female rats after week 39 and of the vehicle control male rats after week 81 was significantly reduced (survival at week 104—male: vehicle control, 29/50; low dose, 33/50; high dose, 40/50; female: 42/50; 40/50; 26/50). Mean body weights of dosed and vehicle control male mice were similar throughout the studies. Mean body weights of high dose female mice were notably lower than those of the vehicle controls after week 28. Survival of the low dose group of male mice was significantly lower than that of vehicle controls at the end of the study (33/50; 24/50; 39/50). No difference in survival was observed between vehicle control and dosed female mice (43/50; 44/50; 43/50).

In the 2-year studies, the kidney was confirmed as the primary target organ for chemically related lesions. No lesions were observed in female rats. For males, the nonneoplastic lesions included exacerbation of the age-related nephropathy, linear deposits of mineral in the renal medulla and papilla, and focal hyperplasia of the transitional epithelium overlying the renal papilla. Uncommon tubular cell adenomas and adenocarcinomas of the kidney also occurred in dosed male rats, and this effect was supported by a dose-related increased incidence of tubular cell hyperplasia, as shown in the table below (see page 4 of the Technical Report).

In subsequent 21-day studies, male and female F344/N rats were administered *d*-limonene at doses ranging from 75 to 1,200 mg/kg. Microscopic examination of the kidney sections from these rats indicated a compound-related increase in intracytoplasmic granules in the proximal convoluted tubules of dosed male rats but not of female rats. The granules were shown to contain $\alpha_2\mu$-globulin by an immunohistochemical strain. $\alpha_2\mu$-Globulin was shown to be increased in kidney homogenates from dosed male rats by an ELISA test.

In mice, no chemically related increases in neoplasms were observed. The incidence of neoplasms of the anterior pituitary gland in high dose female mice was lower than that in vehicle controls (adenomas or carcinomas, combined: vehicle control, 12/49; high dose, 2/48). Cells with an abnormal number of nuclei (8/49; 32/50) and cytomegaly (23/49; 38/50) were observed in the liver of high dose male mice.

Genetic Toxicology: *d*-Limonene was not mutagenic in four strains of *S. typhimurium* (TA98, TA100, TA1535, or TA1537), did not significantly increase the number of trifluorothymidine (Tft)-resistant cells in the mouse L5178Y/TK$^{+/-}$ assay, and did not induce chromosomal aberrations or sister chromatid exchanges (SCEs) in cultured CHO cells. All assays were conducted in the presence and absence of exogenous metabolic activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of *d*-limonene for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney. There was *no evidence of carcinogenic activity* of *d*-limonene for female F344/N rats that received 300 or 600 mg/kg. There was *no evidence of carcinogenic activity* of *d*-limonene for male $B6C3F_1$ mice that received 250 or 500 mg/kg. There was *no evidence of carcinogenic activity* of *d*-limonene for female $B6C3F_1$ mice that received 500 or 1,000 mg/kg.

An increased severity of spontaneous nephropathy, increased incidences of linear mineralization of the renal medulla and papilla, and hyperplasia of the transitional epithelium of the renal papilla were present in dosed male rats.

Synonyms: cyclohexene; 4-isopropenyl-1-methyl; 1-methyl-4-(1-methylethenyl)cyclohexene; *p*-mentha-1,8-diene; carvene; cinene; cajeputene

Report Date: January 1990

TR-348 Toxicology and Carcinogenesis Studies of *alpha*-Methyldopa Sesquihydrate (CAS No. 41372-08-1) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

α-Methyldopa sesquihydrate is used in the treatment of hypertension; over 20 million prescriptions are written annually for α-methyldopa or α-methyldopa sesquihydrate in the United States. α-Methyldopa sesquihydrate (USP grade, greater than 99% pure) was selected for study because of widespread human exposure and the lack of carcinogenicity studies on this compound.

Fourteen-day, 13-week, and 2-year studies were conducted in F344/N rats and $B6C3F_1$ mice. The chemical was administered in feed because human exposure is primarily by the oral route. Short-term studies were performed in bacteria and mammalian cells to evaluate the potential for genetic damage.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, the chemical was administered at dietary concentrations of 0 and 6,250-100,000 ppm. All rats receiving 100,000 ppm and 2/5 female rats receiving 50,000 ppm died. All mice lived until the end of the studies. Final mean body weights of dosed male rats were 14%-43% lower than that of controls, and those of dosed female rats were 9%-24% lower. Feed consumption by dosed male and female rats was reduced. Final mean body weights of dosed mice were generally within 10% of those of controls; feed consumption by dosed groups was lower than that by controls during the first week of the studies.

In the 13-week studies, the chemical was administered at dietary concentrations of 0 and 3,100-50,000 ppm. Deaths occurred in 4/10 male rats, 7/10 female rats, and 2/10 female mice at 50,000 ppm and in 1/10 female rats at 25,000 ppm. Final mean body weights of dosed rats were 6%-46% lower than those of controls. Feed consumption by dosed rat groups was lower than that by controls. Final mean body weights of male mice at 25,000 and 50,000 ppm and female mice at 50,000 ppm were reduced 12%-19%. Feed consumption by dosed and control mice was comparable.

Rats and mice receiving 25,000 and 50,000 ppm exhibited clinical signs of toxicity including lethargy, hyperexcitability, ocular discharge, and rough hair coats. Clinical signs of toxicity were judged to be more severe in dosed male mice than in female mice. Minimal to moderate kidney tubular cell regeneration was seen in male and female rats at 12,500, 25,000, and 50,000 ppm. Bone marrow hypoplasia occurred in male rats at 25,000 and 50,000 ppm and in female rats at 6,300 ppm and higher. Nuclear enlargement (karyomegaly) of the renal cortical tubular epithelium was observed in male and female mice administered 12,500-50,000 ppm; these kidney lesions were judged to be more severe and occurred more frequently at concentrations of 25,000 ppm and higher.

Because of kidney lesions, bone marrow responses, and body weight effects at 12,500 ppm and higher and increased deaths and clinical signs at 25,000 and 50,000 ppm, dietary concentrations selected for male and female rats in the 2-year studies were 0, 3,100, and 6,300 ppm. Based on clinical signs, kidney effects, and body weight decreases at 25,000 and 50,000 ppm, dietary concentrations selected for male and female mice in the 2-year studies were 0, 6,300, and 12,500 ppm. Diets containing the chemical at these concentrations were fed to groups of 50 male and 50 female rats and 50 male and 50 female mice for 103 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were generally 8%-17% lower than those of controls, and mean body weights of dosed mice were generally 5%-22% lower than those of controls throughout the studies. The average amount of α-methyldopa sesquihydrate consumed per day was approximately 110-120 or 230-240 mg/kg per day by low and high dose rats and 830-890 or 1,760-1,800 mg/kg by low and high dose mice. Survival was comparable among dosed and control groups (male rats: control, 28/50; low dose, 26/50; high dose, 27/50; female rats: 35/50; 34/50; 29/50; male mice: 44/50; 42/50; 39/50; female mice: 42/50; 40/50; 38/50). Clinical signs considered to be dose-related included fighting in male rats, irritability in male mice, and rough hair coats in female mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Several lesions of the forestomach, including edema, chronic inflammation, epithelial hyperplasia, and ulcers, were seen at low incidences in high dose rats. No forestomach neoplasms occurred. No neoplastic lesions were observed in either male or female rats which were considered related to α-methyldopa sesquihydrate exposure.

Nephropathy (control, 3/50; low dose, 21/50; high dose, 32/50), karyomegaly (nuclear enlargement) of cells of the tubular epithelium (0/50; 46/50; 44/50, and cysts (2/50; 10/50; 10/50) were observed in the kidney of dosed female mice. Low incidences of tubular cell hyperplasia (0/50; 1/50; 1/50), tubular cell adenomas (0/50; 2/50; 0/50), and tubular cell adenocarcinomas (0/50; 0/50; 1/50) were observed in male mice. Tubular cell adenomas (3/2,029, 0.15%) and tubular cell adenocarcinomas (3/2,029, 0.15%) are uncommon in untreated control male B6C3F$_1$ mice. No neoplastic lesions in female mice were considered related to α-methyldopa sesquihydrate exposure.

Decreased incidences of several site-specific neoplasms were observed in dosed rats and mice; these decreases might have been due in part to decreased weight gain in dosed groups. The decreases occurred in the adrenal medulla of male rats (pheochromocytomas or malignant pheochromocytomas, combined: 21/49; 3/49; 10/50), uterus of female rats (endometrial stromal polyps: 15/50; 5/49; 1/50), liver of male and female mice (hepatocellular adenomas or carcinomas, combined—male: 15/50; 5/50; 6/50; female: 4/50; 1/50; 0/50), and anterior pituitary gland of female mice (adenoma: 9/49; 4/40; 2/50). The incidences of malignant tumors (male: 19/50; 9/50; 8/50; female: 21/50; 16/50; 12/50) and benign or malignant tumors (combined) (male: 32/50; 15/50; 17/50; female: 33/50; 22/50; 21/50) were reduced in dosed mice.

Reproductive Studies: α-Methyldopa sesquihydrate was administered to male F344/N rats in corn oil by gavage 5 days per week for 65 days at doses of 0, 50, 100, 200, or 400 mg/kg. Decreased body weight was seen in dosed animals. Male rats were mated to untreated female F344/N rats on days 57-61, necropsies were performed on days 65-67, and reproductive toxicity was measured by sperm count, sperm motility, organ weights, hormone levels, and histologic evaluation of the testis. Decreased fertility was observed in males dosed with α-methyldopa sesquihydrate at 200 and 400 mg/kg. Decreases were also seen in sperm count, sperm motility, apparent number of late spermatids, and plasma testosterone levels in males in the 200 and 400 mg/kg groups. This alteration of reproductive function in male rats was found to be reversible after a 13-week recovery period (without dosing). The decreased fertility observed after α-methyldopa sesquihydrate administration was probably due in part to the decreases in plasma testosterone levels.

Genetic Toxicity: α-Methyldopa sesquihydrate was not mutagenic when tested with or without exogenous metabolic activation with a preincubation protocol in four strains of *Salmonella typhimurium* (TA97, TA98, TA100, or TA1535). No increase in chromosomal aberrations or sister chromatid exchanges was observed in Chinese hamster ovary (CHO) cells exposed to α-methyldopa sesquihydrate with or without S9.

Audit: The data, documents, and pathology materials from the 2-year studies of α-methyldopa sesquihydrate have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* of α-methyldopa sesquihydrate for male or female F344/N rats fed diets containing 3,100 or 6,300 ppm. There was *equivocal evidence of carcinogenic activity* of α-methyldopa sesquihydrate for male $B6C3F_1$ mice, as shown by three dosed mice having uncommon tubular cell tumors of the kidney. There was *no evidence of carcinogenic activity* of α-methyldopa sesquihydrate for female $B6C3F_1$ mice fed diets containing 6,300 or 12,500 ppm. Nonneoplastic lesions of the kidney including karyomegaly were observed in dosed female mice.

Decreased incidences of several tumor types (in the adrenal gland in male rats, uterus in female rats, liver in male and female mice, and anterior pituitary gland in female mice) were considered related to α-methyldopa sesquihydrate exposure.

Synonyms for α-Methyldopa or α-Methyldopa sesquihydrate: 3-hydroxy-α-methyl-L-tyrosine sesquihydrate; L-(α-MD); α-methyl-L-3,4-dihydroxyphenylalanine; L(-) - β - (3,4-dihydroxyphenyl) - α - methylalanine; L-(-)-3-(3,4-dihydroxyphenyl)-2-methylalanine; L-α-methyl-3,4-dihydroxyphenylalanine; α-methyl-β-(3,4-dihydroxyphenyl)-L-alanine; L-(-)-α-methyl-β-(3,4-dihydroxyphenyl)alanine; (-)-methyldopa; L-methyldopa; L-α-methyldopa; α-methyl-L-dopa

Trade Names for α-Methyldopa or α-Methyldopa sesquihydrate: Aldomet; Aldometil; Aldomin; α-Medopa; AMD; Bayer 1440 L; Baypresol; Dopamet; Dopatec; Dopegyt; Hyperpax; Medomet; Medopren; Methoplain; MK. B51; MK-351; Presinol; Presolisin; Sedometil; Sembrina

Report Date: March 1989

TR-349 Toxicology and Carcinogenesis Studies of Two Pentachlorophenol Technical-Grade Mixtures (CAS No. 87-86-5) in $B6C3F_1$ Mice (Feed Studies)

Toxicology studies of pentachlorophenol, a biocide used primarily as a wood preservative, were conducted by feeding diets containing a technical-grade composite, Dowicide EC-7 (a technical grade formulation), or pure pentachlorophenol to groups of $B6C3F_1$ mice for 30 days. These three grades plus another commercial grade of pentachlorophenol (DP-2) were used in 6-month studies. These studies were followed by 2-year carcinogenicity studies of technical-grade pentachlorophenol and of Dowicide EC-7 in feed. Genetic toxicology studies were conducted in *Salmonella typhimurium* and in Chinese hamster ovary (CHO) cells.

Thirty-Day and Sixteen-Month Studies: Groups of 19 male mice and 5-15 female mice were fed diets containing 0, 20, 100, 500, 2,500, or 12,500 ppm technical-grade pentachlorophenol, Dowicide EC-7, or pure pentachlorophenol for 30 consecutive days. Necropsies and histopathologic examinations were performed on all animals. Selected organs were weighed. Supplemental analyses included hematology, serum chemistry, urinalysis, immunology, and hepatic enzyme induction. Compound-related deaths were observed at the highest dose (12,500 ppm) with all three materials and at 2,500 ppm with EC-7 and pure pentachlorophenol (males only). Decreases in body weight gain were also observed in the groups in which deaths occurred. Diffuse centrilobular cytomegaly, karyomegaly, nuclear atypia, degeneration, or necrosis of the liver were compound-related lesions observed in all groups that received pure pentachlorophenol, technical-grade pentachlorophenol, or EC-7 at 500 ppm and above. Serum enzymes associated with liver injury were increased.

In the 6-month studies, groups of 10 male and 10 female mice were given diets containing the various grades of pentachlorophenol at the following dietary concentrations: 200, 600, or 1,800 ppm technical-grade pentachlorophenol; 200, 600, or 1,200 ppm DP-2 (not used in the 30-day studies); 200, 600, or 1,200 ppm EC-7; or 200, 500, or 1,500 ppm pure pentachlorophenol for 26-27 weeks. Common control groups of 10 male and 10 female mice were fed control diets. Additional groups of male mice were examined for behavioral, histopathologic, clinical pathology, biochemical, and immunologic effects.

All mice exposed at the highest dose of technical-grade pentachlorophenol died, as did 2/10 male mice exposed at the highest dose of DP-2. No deaths were observed in mice exposed to EC-7 or pure pentachlorophenol. Markedly lower final body weights were observed in the high dose groups only (all grades of pentachlorophenol). No chemical-related clinical signs were observed at sublethal doses. No major behavioral changes were observed after 5 weeks' exposure, but increased motor activity and heightened startle responses were present at the end of the study in female mice exposed to all four grades of pentachlorophenol. All grades of pentachlorophenol caused increases in serum enzymes associated with liver injury. All grades of pentachlorophenol also resulted in a dose-related induction of aryl hydrocarbon hydroxylase and an increase in cytochrome P450. However, the technical grade was a more powerful inducer than the other grades of pentachlorophenol. Pure pentachlorophenol had no effect on humoral or cell-mediated immunity. However, DP-2 and particularly technical-grade pentachlorophenol depressed humoral immune function. A

dose-related increase in liver weight was observed in mice exposed to all grades of pentachlorophenol. A dose-related increase in spleen weight was observed in male mice exposed to all grades of pentachlorophenol; a decrease in spleen weight was observed in female mice exposed to all grades of pentachlorophenol except pure.

After 6 months' exposure, histopathologic examination consistently revealed effects in the liver and urinary bladder. The liver lesions were present at all doses with all four grades of pentachlorophenol but were less severe at comparable doses in the mice exposed to pure pentachlorophenol; they consisted of hepatocellular karyomegaly, cytomegaly, and degeneration. The changes in the urinary bladder consisted of a brown granular pigment in the cells of the surface epithelium. No inflammation or proliferative response was associated with the pigment.

Based primarily on the liver lesions observed in the 6-month studies, diets chosen for the 2-year studies contained 0, 100, or 200 ppm technical-grade pentachlorophenol or 0, 100, 200, or 600 ppm EC-7, fed to groups of 50 male and 50 female mice. DP-2 and pure pentachlorophenol were not chosen for the 2-year studies because of economic considerations and because the clinicopathologic syndrome observed in the 6-month studies was similar to that observed with EC-7.

Body Weights and Survival in the Two-Year Studies: Mean body weights of mice exposed to technical-grade pentachlorophenol and EC-7 were comparable to those of controls until weeks 36-82. Thereafter, a 4%-22% dose-related decrease was observed in the mid and high dose mice exposed to EC-7 and in high dose mice exposed to technical-grade pentachlorophenol. Females were more affected than males. Feed consumption by exposed mice was similar to that by controls. The average daily doses of technical-grade pentachlorophenol were approximately 17-18 or 35 mg/kg compared with 17-18, 34-37, or 114-118 mg/kg of EC-7. Survival of mice did not appear to be affected by exposure to either technical-grade pentachlorophenol or EC-7 at the doses used in these studies.

Neoplastic and Nonneoplastic Effects in the Two-Year Studies: The incidences of hepatocellular adenomas and carcinomas were increased (dose related) in male and female mice exposed to either technical-grade pentachlorophenol or EC-7, although the increase was less marked in females exposed to technical-grade pentachlorophenol (adenomas or carcinomas, combined: technical-grade: male – control, 7/32, 22%; low dose, 26/47, 55%; high dose, 37/48, 77%; female – 3/33, 9%; 9/49, 18%; 9/50, 18%; EC-7: male – control, 6/35, 17%; low dose, 19/48, 40%; mid dose, 21/48, 44%; high dose, 34/49, 69%; female – 1/34, 3%; 4/50, 8%; 6/49, 12%; 31/48, 65%).

The incidences of pheochromocytomas in male mice were significantly greater than those in controls for both technical-grade pentachlorophenol (0/31; 10/45, 22%; 23/45, 51%) and EC-7 (1/34, 3%; 4/48, 8%; 21/48, 44%; 45/49, 92%). These neoplasms were also increased in female mice exposed to EC-7 at the highest dose (0/35; 2/49, 4%; 2/46, 4%; 38/49, 78%) but not in those exposed to technical-grade pentachlorophenol (2/33, 6%; 2/48, 4%; 1/49, 2%). Hyperplasia of the adrenal medulla was observed at increased incidences in mice that received either technical-grade pentachlorophenol (male: 1/31; 10/45; female: 0/33; 4/48; 2/49) or EC-7 (male: 1/34; 19/48; 13/48; 1/49; female: 2/35; 1/49; 5/46; 17/49).

The incidences of hemangiosarcomas in the spleen and/or liver were significantly greater than those in controls for high dose female mice that received technical-grade pentachlorophenol (0/35; 3/50, 6%; 6/50, 12%) or EC-7 (0/35; 1/50, 2%; 3/50, 6%; 8/49, 16%).

Compound-related nonneoplastic lesions occurred in the liver, spleen, and nose in mice exposed to either technical-grade pentachlorophenol or EC-7. The lesions in the liver included dose-related increased incidences of clear cell foci, chronic active inflammation, pigmentation, necrosis, cytomegaly, proliferation of hematopoietic cells, and bile duct hyperplasia. Increased amounts of extramedullary hematopoiesis of the splenic red pulp were observed at increased incidences in dosed male and high dose female mice that received technical-grade pentachlorophenol (male: 5/30; 15/23; 18/46; female: 2/33; 4/13; 11/47). Acute focal inflammation of the nasal mucosa and focal metaplasia of the olfactory epithelium were observed at increased incidences in high dose mice that received EC-7 (inflammation – male: 4/35; 1/13; 3/16; 47/49; female: 0/35; 0/14; 2/5; 46/48; focal metaplasia – male: 2/35; 1/13; 2/16; 46/49; female: 1/35; 0/14; 2/5; 45/48) but not in mice exposed to technical-grade pentachlorophenol.

Genetic Toxicology: Pentachlorophenol (91.6% pure; equivalent in purity to the technical-grade pentachlorophenol used in the toxicology studies) was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in the presence or absence of exogenous metabolic activation (S9). In cytogenetic studies with cultured CHO cells, pentachlorophenol produced an increase in chromosomal aberrations in the presence but not the absence of S9 metabolic activation; conversely, sister chromatid exchanges (SCEs) were induced only in the absence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of pentachlorophenol have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity* for male B6C3F$_1$ mice fed diets containing technical-grade pentachlorophenol, as shown by increased incidences of adrenal medullary and hepatocellular neoplasms. There was *some evidence of carcinogenic activity* for female B6C3F$_1$ mice exposed to technical-grade pentachlorophenol, as shown by increased incidences of hemangiosarcomas and hepatocellular neoplasms. There was *clear evidence of carcinogenic activity* for male B6C3F$_1$ mice exposed to pentachlorophenol, EC-7, as shown by increased incidences of adrenal medullary and hepatocellular neo-

plasms. There was *clear evidence of carcinogenic activity* for female $B6C3F_1$ mice exposed to pentachlorophenol, EC-7, as shown by increased incidences of adrenal medullary and hepatocellular neoplasms and hemangiosarcomas.

Chemically related increased incidences of nonneoplastic lesions in mice of each sex included hepatocellular cytomegaly, necrosis, inflammation, pigmentation, and clear cell foci and intrahepatic bile duct hyperplasia.

Synonyms or Common Names: chlorophen; PCP; penchlorol; penta; pentachlorofenol; pentachlorofenolo; pentachlorphenol; 2,3,4,5,6-pentachlorophenol

Trade Names: Acutox; Chem-Penta; Chem-Tol; Cryptogil ol; Dowicide 7; Dowicide EC-7; Dow Pentachlorophenol DP-2 Antimicrobial; Durotox; EP 30; Fungifen; Fungol; Glazd Penta; Grundier Arbezol; Lauxtol; Lauxtol A; Liroprem; Moosuran; Pentacon; Penta-Kil; Pentasol; Penwar; Peratox; Permacide; Permagard; Permasan; Permatox; Priltox; Permite; Santophen; Santophen 20; Sinituho; Term-i-Trol; Thompson's Wood Fix; Weedone; Witophen P

Report Date: March 1989

TR-350 Toxicology and Carcinogenesis Studies of Tribromomethane (Bromoform) (CAS No. 75-25-2) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Tribromomethane, a chemical intermediate and solvent, has been identified as a drinking water contaminant resulting from water chlorination. Toxicology and carcinogenesis studies were conducted by administering tribromomethane (95%-97% pure) in corn oil by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex once or for 14 days, 13 weeks, or 2 years.

Single-Administration, Fourteen-Day, and Thirteen-Week Studies: All rats that received 2,000 mg/kg and 3/5 males and 3/5 females that received 1,000 mg/kg tribromomethane died before the end of the single-administration studies. All mice that received 2,000 mg/kg, 4/5 males and 2/5 females that received 1,000 mg/kg, and 1/5 males that received 500 mg/kg died before the end of the studies. Shallow breathing was observed for rats and male mice that received 1,000 or 2,000 mg/kg tribromomethane.

In the 14-day studies, all rats that received 600 or 800 mg/kg and 1/5 males that received 400 mg/kg tribromomethane died before the end of the studies. The final mean body weight of male rats that received 400 mg/kg was 14% lower than that of vehicle controls. One of five male mice that received 600 mg/kg and 1/5 female mice that received 800 mg/kg died before the end of the studies. Final mean body weights of dosed and vehicle control mice were comparable.

None of the rats died before the end of the 13-week studies (doses ranged from 12 to 200 mg/kg). Final mean body weights were comparable for dosed and vehicle control rats. All male rats that received 100 or 200 mg/kg tribromomethane and all female rats that received 200 mg/kg were lethargic. The incidences of cytoplasmic vacuolization of hepatocytes in dosed male rats were slightly increased compared with that in vehicle controls. The severity of this lesion was increased in the 200 mg/kg group. One of 10 female mice that received 100 mg/kg tribromomethane died before the end of the 13-week studies. The final mean body weight of mice that received 400 mg/kg was 8% lower than that of vehicle controls for males and was comparable to that of vehicle controls for females. Cytoplasmic vacuolization of hepatocytes was observed in the liver of 5/10 male mice that received 200 mg/kg and in 8/10 male mice that received 400 mg/kg tribromomethane.

Based on these results, 2-year studies of tribromomethane were conducted by administering 0, 100, or 200 mg/kg tribromomethane in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 F344/N rats of each sex and 50 female $B6C3F_1$ mice. Male $B6C3F_1$ mice were administered 0, 50, or 100 mg/kg tribromomethane on the same schedule.

Body Weights and Survival in the Two-Year Studies: Mean body weights of high dose male and female rats were 10%-28% lower than those of vehicle controls throughout the second year of the studies. Survival of the high dose group of male rats was significantly lower than that of the vehicle controls after week 91; no significant differences in survival were observed between any groups of female rats (male: vehicle control, 34/50; low dose, 30/50; high dose, 11/50; female: 34/50; 28/50; 28/50). Reduced survival for male rats given 200 mg/kg tribromomethane lowered the sensitivity of this group to detect a carcinogenic response. Mean body weights of dosed and vehicle control male mice were comparable throughout the study. Mean body weights of dosed female mice were 5%-16% lower than those of vehicle controls from week 28 to the end of the study. No significant differences in survival were observed between any groups of male mice; the survival of both dosed groups of female mice was significantly lower than that of the vehicle controls after week 77 (male: 41/50; 37/50; 36/50; female: 25/49; 15/50; 20/50). Reduced survival in all groups of female mice was partly due to a utero-ovarian infection; nonetheless, survival of all groups of female mice was at least 50% by week 92.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Uncommon adenomatous polyps or adenocarcinomas (combined) of the large intestine (colon or rectum) were induced in three male rats (vehicle control, 0/50; low dose, 0/50; high dose, 3/50) and in nine female rats (0/50; 1/50; 8/50); the historical incidence of neoplasms of the large intestine is less than 0.2% in approximately 2,000 corn oil vehicle control male F344/N rats, and none has been observed in approximately 2,000 corn oil vehicle control female F344/N rats. Three of the neoplasms of the large intestine (one in the high dose male rats and two in the high dose female rats) were adenocarcinomas.

Focal or diffuse fatty change of the liver was observed at increased incidences in dosed rats (male: 23/50; 49/50; 50/50; female: 19/50; 39/49; 46/50). Active chronic inflammation was observed at increased incidences in dosed male and high dose female rats (male: 0/50; 29/50; 23/50; female: 9/50; 8/49; 27/50). The incidence of necrosis of the liver was increased in high dose male rats (7/50; 3/50; 20/50) and decreased in dosed females (11/50; 3/49; 2/50).

Mixed cell focus was observed at increased incidences in dosed female rats (8/50; 25/49; 28/50).

Other nonneoplastic lesions observed at increased incidences in dosed rats included chronic active inflammation and squamous metaplasia of the ducts of the salivary gland (squamous metaplasia—male: 0/50; 15/50; 31/48; female: 0/49; 10/49; 16/50; chronic active inflammation—male: 0/50; 16/50; 25/48; female: 0/49; 9/49; 18/50), squamous metaplasia of the prostate gland (2/49; 6/46; 12/50), ulcers of the forestomach (male: 1/49; 5/50; 10/50), and chronic active inflammation of the lung (male: 1/50; 7/50; 15/50). Pigmentation of the spleen was also observed at an increased incidence in high dose female rats. The salivary gland and lung lesions were characteristic of infection by rat coronavirus, a virus to which a positive serologic reaction was observed early in the studies.

The incidence of follicular cell hyperplasia of the thyroid gland was increased in high dose female mice (5/49; 4/49; 19/47), and fatty change of the liver was increased in both dosed groups of female mice (1/49; 9/50; 24/50). No chemically related adverse effects were observed in male mice.

Neoplastic lesions that occurred at lower incidences in dosed animals compared with those in vehicle controls included preputial gland neoplasms in male rats (10/41; 5/38; 1/34), uterine stromal polyps in female rats (10/49; 9/50; 2/50), anterior pituitary gland adenomas in male and female rats (male: 12/50; 12/48; 2/45; female: 29/48; 12/46; 16/48), mammary gland fibroadenomas in female rats (22/50; 17/50; 6/50), and alveolar/bronchiolar neoplasms in male mice (11/50; 7/50; 2/49). Other than concomitant decreases in body weights, no other reasons are obvious to correlate these decreases with chemical administration.

Genetic Toxicology: Tribromomethane exhibited equivocal mutagenicity in *Salmonella typhimurium* strain TA100 in the absence of exogenous metabolic activation and in strains TA97 and TA98 when exposure occurred in the presence of hamster S9; tribromomethane produced no increases in revertant colonies in TA1535 or TA1537 with or without exogenous metabolic activation. Tribromomethane induced trifluorothymidine (Tft) resistance in mouse L5178Y cells with and without metabolic activation. When tested in cultured Chinese hamster ovary (CHO) cells for cytogenetic effects, tribromomethane produced an increase in both sister chromatid exchanges (SCEs) and chromosomal aberrations in the absence, but not in the presence, of exogenous metabolic activation. Tribromomethane caused sex-linked recessive lethal mutations in Drosophila when administered to adult males by feeding; no induction of mutations was observed when tribromomethane was administered by abdominal injection. Results of tests for reciprocal translocations in adult male Drosophila exposed to tribromomethane by feeding were negative. In vivo tests for cytogenetic effects in bone marrow cells of male $B6C3F_1$ mice demonstrated that intraperitoneal injection of tribromomethane induced an increase in SCEs but no increase in chromosomal aberrations. Intraperitoneal injection of tribromomethane also induced an increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow of $B6C3F_1$ mice.

Audit: The data, documents, and pathology materials from the 2-year studies of tribromomethane have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of tribromomethane for male F344/N rats and *clear evidence of carcinogenic activity* for female F344/N rats, based on increased incidences of uncommon neoplasms of the large intestine. Reduced survival for male rats given 200 mg/kg tribromomethane lowered the sensitivity of this group to detect a carcinogenic response. Chemically related nonneoplastic lesions included fatty change and active chronic inflammation of the liver in male and female rats, minimal necrosis of the liver in male rats, and mixed cell foci of the liver in female rats. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice given 50 or 100 mg/kg tribromomethane or for female $B6C3F_1$ mice given 100 or 200 mg/kg; male mice might have been able to tolerate a higher dose. Survival of female mice was reduced, partly due to a utero-ovarian infection.

Synonym: bromoform

Report Date: May 1989

TR-351 Toxicology and Carcinogenesis Studies of *para*-Chloroaniline Hydrochloride (CAS No. 20265-96-7) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

p-Chloroaniline has a large production volume and is used as a dye intermediate. Toxicology and carcinogenesis studies of *p*-chloroaniline (greater than 99% pure) were conducted by administering *p*-chloroaniline hydrochloride in water by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Vehicle controls were given deionized water by gavage. All doses were calculated as *p*-chloroaniline; the chemical was administered as the hydrochloride after dissolution in water containing molar equivalents of hydrochloric acid. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells. Hematologic parameters were measured at the end of the

13-week studies and at 6, 12, 18, and 24 months in the 2-year studies. Supplemental studies of the distribution and disposition of *p*-chloroaniline were conducted in male F344 rats.

Sixteen-Day and Thirteen-Week Studies: In the 16-day studies, male and female rats and mice received 25, 50, 100, or 400 mg/kg of body weight. The vehicle controls received deionized water. All rats and mice that received 200 or 400 mg/kg died during the first 6 days of the studies. Some deaths occurred in each of the lower dose groups of mice. Splenic enlargement was observed at necropsy in rats administered 25, 50, or 100 mg/kg. Congestion of the spleen and hemosiderin deposition in the renal cortical tubular epithelial cells were observed at 100 mg/kg in male and female rats. Compound-related lesions in mice included hemosiderosis of the liver Kupffer cells and congestion of the spleen.

In the 13-week studies, 10 rats of each sex were administered doses of 0, 5, 10, 20, 40, or 80 mg/kg. All male rats lived to the end of the 13-week studies. One of 10 female rats that received 80 mg/kg died from unknown causes. The final mean body weights of rats that received 80 mg/kg were 16% lower than that of vehicle controls for males and 4% lower for females. In the 13-week studies in mice, 10 animals of each sex were administered doses of 0, 7.5, 15, 30, 60, or 120 mg/kg. Deaths in mice were not related to *p*-chloroaniline hydrochloride administration. The final mean body weights of dosed and vehicle control mice were similar. In both rats and mice, no chemically related effects on organ weights were observed at necropsy, except for the spleen, which was enlarged as a function of increasing dose. Methemoglobin was increased in dosed groups and resulted in a secondary anemia, the severity of which was dose related. Compound-related lesions observed histologically, including pigmentation (hemosiderin) in the kidney, spleen, and liver and hematopoiesis in the liver and spleen, reflected the response to the hemolytic anemia and methemoglobinemia induced by *p*-chloroaniline hydrochloride.

Based on these results, groups of 50 rats of each sex were administered 2, 6, or 18 mg/kg *p*-chloroaniline hydrochloride in water by gavage, 5 days per week for 103 weeks. Groups of 50 mice of each sex were administered 3, 10, or 30 mg/kg on the same schedule.

Metabolism and Disposition Studies in Rats: The metabolism and disposition studies in F344/N rats showed that metabolic and excretory pathways were not saturated by *p*-chloroaniline administered orally at doses ranging from 0.3 to 30 mg/kg. *p*-Chloroaniline was rapidly metabolized and excreted primarily in urine with a half-life of approximately 2 hours.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were generally within 5% of those of vehicle controls throughout the studies. The survival of the low and mid dose groups of male rats and of the low and high dose groups of female rats was significantly greater than that of the vehicle controls (male: vehicle control, 18/49; low dose, 32/50; mid dose, 32/50; high dose, 21/50; female: 27/50; 39/50; 36/50; 37/50). The increased survival was attributed to the decreased incidences of mononuclear cell leukemia. Mean body weights of high dose male and female mice were generally within 5% of those of vehicle controls throughout the studies. The survival of the mid dose group of male mice was lower than that of the vehicle controls after week 99 (male: 43/50; 36/50; 29/50; 35/50; female: 39/50; 42/50; 44/50; 41/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Fibrosis of the spleen was increased in dosed male and high dose female rats (male: vehicle control, 3/49; low dose, 11/50; mid dose, 12/50; high dose, 41/50; female: 1/50; 2/50; 3/50; 42/50). Cellular infiltration of lipocytes (fatty metaplasia) was observed in the spleen at increased incidences in high dose rats (male: 0/49; 0/50; 0/50; 24/50; female: 0/50; 0/50; 0/50; 11/50). The incidence of uncommon sarcomas of the spleen in high dose male rats was significantly greater than that in the vehicle controls (fibrosarcomas, osteosarcomas, or hemangiosarcomas, combined: 0/49; 1/50; 3/50; 38/50). Many of these tumors metastasized to one or more sites. In female rats, one fibrosarcoma of the spleen was found in a mid dose animal, and one osteosarcoma of the spleen was found in a high dose animal. The historical incidence of splenic connective tissue sarcomas (all types) in water gavage vehicle controls is 1/298 (0.3%) for male rats and 0/297 for female rats. The historical incidence of hemangiosarcomas in water gavage controls is 0/300 for male rats and 1/297 (0.3%) for female rats.

Adrenal medullary hyperplasia was observed at an increased incidence in high dose female rats (4/50; 4/50; 7/50; 24/50). Marginally increased incidences of pheochromocytomas were seen in high dose male (13/49; 14/48; 15/48; 26/49) and female (2/50; 3/50; 1/50; 6/50) rats. The historical incidence of pheochromocytomas in water gavage vehicle control male F344/N rats is 121/299 (40% ± 16%); the historical incidence in water gavage vehicle control female F344/N rats is 20/295 (7% ± 2%).

The incidences of mononuclear cell leukemia in dosed male and female rats were lower than those in vehicle controls (male: 21/49; 3/50; 2/50; 3/50; female: 10/50; 2/50; 1/50; 1/50). The incidences of malignant lymphomas in dosed male and female mice were lower than those in vehicle controls (male: 10/50; 3/49; 9/50; 3/50; female: 19/50; 12/50; 5/50; 10/50).

Hematologic and methemoglobin measurements were made on blood samples collected from 15 randomly selected male and female rats per dose group at 6, 12, 18, and 24 months. In general, the high dose group at various intervals showed mild hemolytic anemia and dose-related increases in methemoglobin.

In rats, compound-related nonneoplastic lesions were seen histopathologically in the bone marrow, spleen, and liver. These lesions included bone marrow hyperplasia, hepatic hemosiderosis, and splenic fibrosis and suggest compound-related effects on the hematopoietic system in general, the erythropoietic system specifically, and mesenchymal cells in the spleen.

In male mice, the incidence of hemangiosarcomas of the liver or spleen in high dose male mice was greater than that

in the vehicle controls (4/50; 4/49; 1/50; 10/50). The historical incidence of hemangiomas or hemangiosarcomas at all sites (combined) in water gavage vehicle control male $B6C3F_1$ mice is 11/350 (3% ± 3%).

The incidences of hepatocellular adenomas or carcinomas (combined) were increased in dosed male mice (11/50; 21/49; 20/50; 21/50), primarily due to increased incidences of hepatocellular carcinomas (3/50; 7/49; 11/50; 17/50). Hepatocellular carcinomas metastasized to the lung in 1/50 vehicle control, 1/49 low dose, 2/50 mid dose, and 9/50 high dose male mice. The historical incidence of hepatocellular neoplasms in water gavage vehicle controls is 106/347 (31 ± 6%).

Genetic Toxicology: *p*-Chloroaniline was mutagenic in *S. typhimurium* strains TA98 and TA100 in the presence of exogenous metabolic activation; no increase in revertant colonies was observed in strains TA97, TA1535, or TA1537. *p*-Chloroaniline induced trifluorothymidine (Tft) resistance in mouse L5178Y lymphoma cells with and without metabolic activation. In cultured CHO cells, treatment with *p*-chloroaniline produced significant increases in sister chromatid exchanges (SCEs) both with and without metabolic activation (S9); chromosomal aberrations were significantly increased only in the presence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of *p*-chloroaniline have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year water gavage studies, there was *clear evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for male F344/N rats, as indicated by increased incidences of uncommon sarcomas of the spleen. Pheochromocytomas of the adrenal gland may also have been associated with chemical administration. There was *equivocal evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for female F344/N rats, as indicated by the presence of uncommon sarcomas of the spleen in one mid and one high dose animal and the increased incidence of pheochromocytomas of the adrenal gland. There was *some evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for male $B6C3F_1$ mice, as indicated by increased incidences of hepatocellular neoplasms and of hemangiosarcomas of the liver or spleen. There was *no evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for female $B6C3F_1$ mice administered 3, 10, or 30 mg/kg by gavage for 2 years.

The incidences of mononuclear cell leukemia in male and female rats and of malignant lymphomas in male and female mice were decreased by administration of *p*-chloroaniline hydrochloride. Compound-related splenic fibrosis was present in male and female rats.

Synonyms: 1-amino-4-chlorobenzene hydrochloride; 4-chlorophenylamine hydrochloride; 4-chlorobenzeneamine hydrochloride

Report Date: July 1989

TR-352 Toxicology and Carcinogenesis Studies of *N*-Methylolacrylamide (CAS No. 924-42-5) in F344/N Rats and $B6C3F_1$ Mice

N-Methylolacrylamide is a cross-linking agent used in adhesives, binders for paper, crease-resistant textiles, resins, latex film, and sizing agents. Toxicology and carcinogenesis studies were conducted by administering *N*-methylolacrylamide (98% pure) in water by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 16 days, 13 weeks, or 2 years. In vitro genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells; an in vivo bone marrow micronucleus test was performed with $B6C3F_1$ mice. Neurobehavioral assays were performed during the 13-week studies.

Sixteen-Day Studies: The doses of *N*-methylolacrylamide used ranged from 25 to 400 mg/kg. All rats that received 400 mg/kg died within 4 days, and 3/5 male rats that received 200 mg/kg also died before the end of the studies. Compound-related clinical signs seen with 200 mg/kg included ataxia, muscle tremors, and hyperirritability. Ataxia after dosing was observed from day 7 to the end of the studies for rats that received 100 mg/kg. The final mean body weight of male rats that received 100 or 200 mg/kg was 10% or 27% lower than that of the vehicle controls. The final mean body weight of female rats that received 200 mg/kg was 20% lower than that of the vehicle controls. Compound-related lesions in rats included hyperplasia of the bronchiolar and tracheal epithelium, dysplasia of the nasal and tracheal epithelium, centrilobular hepatocellular necrosis, lymphoid depletion of the spleen, and myelin degeneration of the lumbar ventral spinal nerve.

All 5 male and 4/5 female mice that received 400 mg/kg *N*-methylolacrylamide died on the second day of the 16-day studies. The surviving female mouse in the 400 mg/kg group and the male and female mice in the 200 mg/kg groups were ataxic after they were dosed, starting on day 2. Weight changes were inconsistent among dose groups. Bronchial epithelial hyperplasia (mild) appeared to be dose related in males and females. Sinusoidal congestion of the liver and vacuolar degeneration of myocardial fibers were seen in males and females given 400 mg/kg.

Thirteen-Week Studies: The doses of *N*-methylolacrylamide used ranged from 12.5 to 200 mg/kg. All rats that received 100 or 200 mg/kg died before the end of the studies. Rats that received 100 or 200 mg/kg had hind limb ataxia, which progressed to hind limb paralysis. Rats that received 50 mg/kg had hind limb ataxia beginning at week 8, which progressed to hind limb paresis by week 11. The final mean body weight of rats that received 25 or 50 mg/kg was 8% or 16% lower than that of the vehicle controls for males and 6% or 10% lower for females. In neurobehavioral assessments, decreased forelimb and hind limb grip strength was seen at doses as low as 25 mg/kg for female rats and at doses as low as 12.5 mg/kg for male rats. A decreased startle response was seen for females at doses as low as 25 mg/kg. The landing

foot spread was significantly increased for male and female rats that received 50 mg/kg.

Axon filament and myelin sheath degeneration of the brain stem, spinal cord, and/or peripheral nerves was seen in rats at increased incidences at 25 mg/kg and higher doses. Inflammation and/or hemorrhage and edema of the urinary bladder mucosa were seen with doses of 25 mg/kg or more in a few rats that had distended bladders at gross examination.

All mice that received 200 mg/kg *N*-methylolacrylamide died before the end of the studies. Final mean body weights of dosed and vehicle control mice were similar. A decreased relative testis weight was observed for mice that received 12.5 mg/kg or more. The relative kidney weights for male mice receiving 50 or 100 mg/kg were greater than that for vehicle controls. Neurobehavioral studies indicated decreased forelimb grip strength in male and female mice at doses as low as 25 mg/kg. An exaggerated startle response was seen for female mice given 100 mg/kg. A reduction in rotarod performance was seen for male and female mice receiving 100 mg/kg and for male mice receiving 25 mg/kg.

Hepatocellular necrosis and thymic lymphocytic necrosis were compound-related effects in mice given 200 mg/kg *N*-methylolacrylamide. Hemorrhage, necrosis, and mineralization of the zona reticularis of the adrenal gland were present in 3/10 female mice given 200 mg/kg, and cytoplasmic vacuolization of the adrenal cortex was seen with lower doses.

Based on the results of these short-term studies, 2-year studies were conducted by administering 0, 6, or 12 mg/kg *N*-methylolacrylamide in water by gavage, 5 days per week for 103 weeks, to groups of 50 rats of each sex. Groups of 50 mice of each sex were administered 0, 25, or 50 mg/kg on the same schedule.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were within 6% of those of vehicle controls throughout most of the studies. Mean body weights of dosed mice were as much as 25% greater than those of vehicle controls for females and as much as 13% greater for males. The survival of female rats given 25 mg/kg per day was lower than that of vehicle controls after day 550, but survival of female rats given 50 mg/kg per day was not different from that of vehicle controls (vehicle control, 35/50; low dose, 22/50; high dose, 33/50). No differences in survival were observed between any other groups of rats or mice of either sex (male rats: 28/50; 22/50; 27/50; male mice: 30/50; 20/50; 21/50; female mice: 41/50; 35/50; 33/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: In rats, no biologically important nonneoplastic or neoplastic lesions were attributed to administration of *N*-methylolacrylamide. Higher doses might have increased the sensitivity of the studies to determine the presence or absence of a carcinogenic response.

In mice, the incidences of adenomas of the Harderian gland were increased in males given either dose of *N*-methylolacrylamide and in females given the top dose (male: vehicle control, 1/48; low dose, 14/49; high dose, 29/50; female: 5/47; 8/45; 20/48). The incidences of carcinomas of the Harderian gland were not significantly increased by *N*-methylolacrylamide administration (male: 1/48; 0/49; 2/50; female: 0/47; 3/45; 2/48).

The incidences of hepatocellular adenomas were increased in male and female mice given 50 mg/kg *N*-methylolacrylamide (male: 8/50; 4/50; 19/50; female: 3/50; 4/50; 17/49). The incidences of hepatocellular carcinomas were also marginally increased in dosed male mice (male: 6/50; 13/50; 12/50; female: 3/50; 3/50; 2/49). Hepatocellular adenomas and carcinomas (combined) occurred with positive trends, and the incidences in male and female mice receiving 50 mg/kg were increased compared with those in the vehicle controls (male: 12/50; 17/50; 26/50; female: 6/50; 7/50; 17/49).

Chronic inflammation and alveolar epithelial hyperplasia of the lung were observed at increased incidences in mice given *N*-methylolacrylamide. Sentinel mice were seropositive for Sendai virus at 18 months. The incidences of alveolar/bronchiolar adenomas (3/49; 6/50; 11/50) and carcinomas (2/49; 4/50; 10/50) were increased in male mice given 50 mg/kg. Alveolar/bronchiolar adenomas or carcinomas (combined) occurred with a positive trend in male mice (5/49; 10/50; 18/50). The incidence of alveolar/bronchiolar adenomas or carcinomas (combined) was increased in female mice given the top dose of 50 mg/kg (6/50; 8/50; 13/49).

Ovarian atrophy was observed at increased incidences in female mice receiving *N*-methylolacrylamide (3/50; 39/45; 38/47). The incidences of benign granulosa cell tumors were also increased in the dosed groups (0/50; 5/45; 5/47).

The incidence of adenomas of the pars distalis in high dose female mice was significantly lower than that in vehicle controls (13/49; 5/14; 4/43).

Genetic Toxicology: *N*-Methylolacrylamide was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535 when tested with or without exogenous metabolic activation. *N*-Methylolacrylamide induced both sister chromatid exchanges (SCEs) and chromosomal aberrations in CHO cells with and without metabolic activation. No increase in micronucleated polychromatic erythrocytes (PCEs) was observed in the bone marrow of $B6C3F_1$ mice after intraperitoneal injection of *N*-methylolacrylamide.

Conclusions: Under the conditions of these 2-year studies, there was *no evidence of carcinogenic activity* of *N*-methylolacrylamide for male or female F344/N rats receiving doses of 6 or 12 mg/kg per day by aqueous gavage. There was *clear evidence of carcinogenic activity* of *N*-methylolacrylamide for male $B6C3F_1$ mice, based on increased incidences of neoplasms of the Harderian gland, liver, and lung. There was *clear evidence of carcinogenic activity* of *N*-methylolacrylamide for female $B6C3F_1$ mice, based on increased incidences of neoplasms of the Harderian gland, liver, lung, and ovary.

In rats, because no biologically important toxic effects were attributed to *N*-methylolacrylamide administration, somewhat higher doses could have been used to increase the sensitivity of these studies for determining the pres-

ence or absence of a carcinogenic response. In female mice, ovarian atrophy was compound related.

Synonyms: *N*-(hydroxymethyl)acrylamide; *N*-(hydroxymethyl)-2-propenamide; *N*-methanolacrylamide; monomethylolacrylamide

Report Date: September 1989

TR-353 Toxicology and Carcinogenesis Studies of 2,4-Dichlorophenol (CAS No. 120-83-2) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

2,4-Dichlorophenol is a chemical intermediate used principally in the manufacture of the herbicide 2,4-dichlorophenoxyacetic acid. Toxicology and carcinogenesis studies were conducted by feeding diets containing 2,4-dichlorophenol (greater than 99% pure) for 14 days, 13 weeks, or 2 years to groups of F344/N rats and B6C3F$_1$ mice of each sex. Genetic toxicology tests were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, male and female rats and mice were given diets containing 2,4-dichlorophenol at concentrations up to 40,000 ppm. One high dose male mouse died before the end of the studies; no deaths occurred in any other group, and no compound-related lesions were seen at necropsy in rats or mice. In the 13-week studies, groups of 10 rats and 10 mice of each sex were fed diets containing 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm 2,4-dichlorophenol. All rats lived to the end of the studies, whereas all mice that received 40,000 ppm died during the first 3 weeks of the studies. Final mean body weights of rats that received 20,000 or 40,000 ppm and of male mice that received 20,000 ppm were at least 10% lower than those of controls. Bone marrow atrophy in rats and necrosis and syncytial alteration (multinucleated hepatocytes) in the liver of male mice were compound-related effects. Two-year studies were conducted by feeding diets containing 0, 5,000, or 10,000 ppm 2,4-dichlorophenol to groups of 50 male rats and 50 male and 50 female mice for 103 weeks. Groups of 50 female rats received diets containing 0, 2,500, or 5,000 ppm.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male and female rats, high dose male mice, and both dosed groups of female mice were generally lower than those of controls. No significant differences in survival were observed between any groups of rats or mice of either sex (male rats: control, 33/50; low dose, 25/50; high dose, 32/50; female rats: 34/50; 43/50; 40/50; male mice: 33/50; 32/50; 31/50; female mice: 45/50; 40/50; 43/50). The average daily feed consumption by rats in the low dose and high dose groups was 94%-97% that by the controls. The estimated daily mean consumption of 2,4-dichlorophenol was 210 or 440 mg/kg for low dose or high dose male rats and 120 or 250 mg/kg for low dose or high dose female rats. The average daily feed consumption by mice in the low dose and high dose groups was 97% and 78% of that by the controls for males and 94% and 85% for females. The estimated daily mean consumption of 2,4-dichlorophenol was 800 or 1,300 mg/kg for low dose or high dose male mice and 430 or 820 mg/kg for low dose or high dose female mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: There were no compound-related increased incidences of neoplastic lesions in rats or mice. The incidence of mononuclear cell leukemia was decreased in dosed male rats relative to that in controls (control, 31/50; low dose, 17/50; high dose, 17/50); the incidence of malignant lymphomas was decreased in high dose female mice (4/50) relative to that in controls (12/50). Syncytial alteration of hepatocytes was observed at increased incidences in dosed male mice (11/50; 33/49; 42/48).

Genetic Toxicology: The mutagenic effect of 2,4-dichlorophenol in *S. typhimurium* strain TA1535 was considered to be equivocal only in the presence of hamster S9; 2,4-dichlorophenol produced no increases in revertant colonies in strains TA98, TA100, or TA1537 with or without exogenous metabolic activation. 2,4-Dichlorophenol increased trifluorothymidine (Tft) resistance in the mouse L5178Y assay without metabolic activation; it was not tested with activation. In cultured CHO cells, 2,4-dichlorophenol did not induce chromosomal aberrations but did significantly increase the frequency of sister chromatid exchanges (SCEs) both in the presence and absence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of 2,4-dichlorophenol have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* for male F344/N rats fed diets containing 5,000 or 10,000 ppm 2,4-dichlorophenol or for female F344/N rats fed diets containing 2,500 or 5,000 ppm 2,4-dichlorophenol. There was *no evidence of carcinogenic activity* for male or female B6C3F$_1$ mice fed diets containing 5,000 or 10,000 ppm 2,4-dichlorophenol.

Synonyms: 2,4-DCP; 2,4-dichlorohydroxybenzene

Report Date: June 1989

TR-354 Toxicology and Carcinogenesis Studies of Dimethoxane (CAS No. 828-00-2) (Commercial Grade) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Dimethoxane is used as an antimicrobial agent in water-based paints, dyestuffs, fabric softeners, sizings, and spinning emulsions. In the past, it was used in lipsticks and other cosmetic preparations. Toxicology and carcinogenesis studies were conducted by administering

commercial-grade dimethoxane (80% pure, none of these impurities exceeded 3%) in corn oil gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex one time or 5 days per week for 16 days, 13 weeks, 15 months, or 2 years. Clinical pathology analyses were performed at 15 months in the 2-year studies. Commercial-grade dimethoxane was studied because that is the grade to which humans are generally exposed. The same lot of commercial-grade dimethoxane was used in genetic toxicology tests for mutagenicity in *Salmonella typhimurium*, for sister chromatid exchanges (SCEs) and chromosomal aberrations in Chinese hamster ovary (CHO) cells, and for sex-linked recessive lethal mutations and translocation in Drosophila.

Sixteen-Day Studies: In the 16-day studies, rats and mice received 0, 125, 250, 500, 1,000, or 2,000 mg/kg dimethoxane in corn oil per day. Deaths occurred in rats and in male mice that received 2,000 mg/kg. Body weights of rats and mice were similar to those of vehicle controls. Compound-related clinical signs were not seen in surviving rats. Hemorrhage and necrosis of the stomach were observed in rats in the 2,000 mg/kg group which died before the end of the studies. Lesions of the forestomach, including inflammation, hyperplasia, hyperkeratosis, and ulceration, occurred in rats that received 250-2,000 mg/kg. Mice that received 500-2,000 mg/kg dimethoxane had lesions of the forestomach including erosion, ulceration, hyperplasia, and hyperkeratosis. Forestomach lesions were not seen at 125 or 250 mg/kg.

Thirteen-Week Studies: No compound-related deaths occurred in rats. Doses used were 0, 31, 62, 125, 250, or 500 mg/kg dimethoxane in corn oil by gavage. The final mean body weights of rats that received 500 mg/kg were 17% lower than that of vehicle controls for males and 5% lower for females. Ulceration, inflammation, and acanthosis with hyperkeratosis of the stratified squamous epithelium of the forestomach were seen in rats that received 500 mg/kg. Forestomach lesions were not seen in males that received 31 mg/kg or in females that received 31, 62, or 125 mg/kg.

All mice lived to the end of the studies (doses used were 0, 31, 62, 125, 250, or 500 mg/kg dimethoxane in corn oil by gavage). Final mean body weights of dosed and vehicle control mice were similar. Minimal-to-mild acanthosis and hyperkeratosis of the squamous epithelium of the forestomach were seen in 4/10 high dose male and 1/10 high dose female mice.

Because of the forestomach lesions observed in rats and mice and reduced body weight observed for male rats, doses selected for the 2-year studies were 0, 62.5, or 125 mg/kg dimethoxane in corn oil, given by gavage 5 days per week to groups of 60 male rats; 0, 125, or 250 mg/kg to groups of 60 female rats; and 0, 250, or 500 mg/kg to groups of 58 or 60 mice of each sex. Ten animals per sex and species from each dose group were killed 15 months after initiation of the studies to determine toxicity, preneoplastic lesions, and early induced neoplasia.

Fifteen-Month Studies: Minimal diffuse acanthosis and hyperplasia of the forestomach were seen in 7/10 female rats at 250 mg/kg, 7/10 males at 125 mg/kg, and 1/9 male and 1/9 female vehicle controls. Acanthosis of the forestomach was seen in 7/10 male and 6/10 female mice at 500 mg/kg. Harderian gland adenomas were seen in one high dose male and one high dose female mouse. A harderian gland adenocarcinoma was seen in a second high dose female mouse. No compound-related effects were observed for clinical chemical or hematologic values or for organ weights for rats or mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed and vehicle control rats and mice of each sex were generally similar. No significant differences in survival were observed between any groups of rats (male: vehicle control, 23/50; low dose, 28/50; high dose, 21/50; female: 30/50; 31/50; 24/50) or mice (male: 33/50; 27/48; 29/50; female: 36/50; 35/50; 34/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: At no site was a significantly increased incidence of neoplastic lesions observed in dosed male or female rats or in dosed female mice. Acanthosis and hyperkeratosis were increased in the forestomach of high dose rats; acanthosis, hyperkeratosis, focal hyperplasia, and chronic active inflammation were increased in the forestomach of dosed mice. The incidence of squamous cell papillomas of the forestomach was increased in high dose male mice (vehicle control, 2/47; low dose, 3/47; high dose, 7/50). A squamous cell carcinoma of the forestomach was present in another high dose male mouse. Although the incidence of squamous cell papillomas in the high dose group was not significantly different from that in the vehicle controls, the incidence exceeded the highest observed in historical corn oil gavage vehicle controls (3/49). Other than a single squamous cell papilloma in the esophagus of a low dose male mouse, no hyperplastic or neoplastic lesions were seen outside the stomach of dosed mice which could be related to the administration of dimethoxane. Despite the observation of three harderian gland neoplasms in mice killed at 15 months, no increase in the incidences of harderian gland neoplasms was seen in dosed mice in the 2-year studies (male: 2/48; 2/48; 2/48; female: 2/48; 0/49; 2/50).

Genetic Toxicity: Dimethoxane was mutagenic in strain TA100 of *S. typhimurium* in the presence but not the absence of exogenous metabolic activation; it was not mutagenic in strains TA98, TA1535, or TA1537 with or without activation. Dimethoxane induced SCEs and chromosomal aberrations in CHO cells both with or without exogenous metabolic activation. Dimethoxane induced sex-linked recessive lethal mutations in Drosophila when administered by abdominal injection to adult males; no induction of reciprocal translocations was observed in adult males after injection of dimethoxane.Conclusions: Under the conditions of these 2-year corn oil gavage studies, there was *no evidence of carcinogenic activity* of dimethoxane for male F344/N rats receiving 62.5 or 125 mg/kg or for female F344/N rats receiving 125 or 250 mg/kg per day. There was *equivocal evidence of carcinogenic activity* of dimethoxane for male B6C3F$_1$ mice, as indicated by an increased incidence of fore-

stomach neoplasms. There was *no evidence of carcinogenic activity* for female B6C3F$_1$ mice receiving 250 or 500 mg/kg per day. Acanthosis and hyperkeratosis occurred at increased incidences in the forestomach of high dose rats. Inflammation, acanthosis with hyperkeratosis, and focal hyperplasia occurred at increased incidences in the forestomach of dosed mice.

Synonyms: acetomethoxan; acetomethoxane; 6-acetoxy-2,4-dimethyl-*m*-dioxane; 2,6-dimethyl-*m*-dioxan-4-yl acetate; 2,6-dimethyl-*m*-dioxan-4-ol acetate; 2,6-dimethyl-1,3-dioxan-4-ol acetate

Report Date: September 1989

TR-355 Toxicology and Carcinogenesis Studies of Diphenhydramine Hydrochloride (CAS No. 147-24-0) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Diphenhydramine hydrochloride is a widely used antihistaminic drug in human and veterinary medicine. Toxicology and carcinogenesis studies were conducted by feeding diets containing USP-grade diphenhydramine hydrochloride (greater than 99% pure) to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, dietary concentrations ranged from 620 to 10,000 ppm for rats and from 310 to 5,000 ppm for mice. All rats that received diets containing 10,000 ppm and 9/10 rats that received diets containing 5,000 ppm died before the end of the studies. The final mean body weights of rats receiving 1,250 or 2,500 ppm were 12%-13% or 30%-34% lower than those of controls. Feed consumption by rats at the three highest concentrations was more than 30% less than that by controls. All mice receiving 5,000 ppm, 4/5 males and 4/5 females receiving 2,500 ppm, and 4/5 males receiving 1,250 ppm died before the end of the studies. The final mean body weights of mice that received 1,250 or 2,500 ppm were lower than the initial weights. All dosed rats and mice were hyperactive and sensitive to sound and/or touch.

In the 13-week studies, dietary concentrations of diphenhydramine hydrochloride ranged from 156 to 2,500 ppm for rats and from 78 to 1,250 ppm for mice. All rats lived to the end of the studies. The final mean body weights of rats receiving 1,250 or 2,500 ppm were about 15% or 35% lower than those of controls. The final mean body weight of female rats receiving 625 ppm was 9% lower than that of controls. Increased activity was observed for all male and female rats receiving 1,250 and 2,500 ppm. Cytoplasmic vacuolization of the liver, characteristic of fat accumulation, was observed in male and female rats receiving 313-2,500 ppm. The severity of this change increased with increased dose. For mice, 1/10 males receiving 313 ppm, 2/10 males receiving 625 ppm, and 8/10 males receiving 1,250 ppm died before the end of the studies. The final mean body weights of mice that received 625 or 1,250 ppm were about 9% or 16% lower than those of controls. No compound-related histopathological effects were observed in mice.

Based on the mortality and body weight effects of diphenhydramine hydrochloride in the short-term studies, dietary concentrations selected for the 2-year studies were 0, 313, and 635 ppm diphenhydramine hydrochloride for male rats and 0, 156, and 313 ppm for female rats and male and female mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed and control rats were similar throughout the studies, and mean body weights of dosed mice were 3%-13% lower than those of controls throughout most of the studies. No significant differences in survival were observed between any groups of rats or mice of either sex (male rats: control, 29/50; low dose, 32/50; high dose, 24/50; female rats: 35/50; 32/50; 36/50; male mice: 29/50; 30/50; 24/48; female mice: 37/50; 39/50; 32/50). The estimated average daily feed consumption by dosed rats and dosed mice was similar to that by controls. The average amount of diphenhydramine hydrochloride consumed per day was approximately 13 or 27 mg/kg for low dose or high dose male rats, 7 or 15 mg/kg for low dose or high dose female rats, and 21 or 46-47 mg/kg for low dose or high dose male and female mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: For three high dose male rats, astrocytomas were found in brain sections taken by routine sampling procedures. Gliomas, containing neoplastic astrocytes and oligodendrocytes, were found in one control and one additional high dose male rat. The incidence of glial cell tumors in high dose male rats (4/50) exceeded the highest incidence in historical controls in the Program (2/50). The historical incidence of glial cell tumors is less than 0.7% in approximately 2,000 untreated control male F344/N rats. Three additional sections of brain were prepared from the residual fixed tissues of each male and female rat. One additional astrocytoma in a high dose male rat and one astrocytoma in a high dose female rat were observed in these sections.

Adenomas of the anterior pituitary gland in female rats occurred with a significant positive trend; the incidences in low dose male and high dose female rats were marginally greater than those in controls (male: control, 11/49; low dose, 21/50; high dose, 14/49; female: 23/50; 26/50; 35/50).

The incidence of alveolar/bronchiolar adenomas in low dose male rats was slightly greater than that in controls (0/49; 5/50; 3/50). The incidences of alveolar/bronchiolar adenomas or carcinomas (combined) in dosed male rats were not significantly different from that in controls (1/49; 6/50; 5/50) but exceeded the highest incidence in historical controls (4/49). The historical incidence of alveolar/bronchiolar neoplasms in untreated control male F344/N rats is approximately 2.2%. Adenomatous hyperplasia of the lung was not increased in incidence in dosed male rats compared with controls.

The incidences of granulomas of the liver were increased in dosed rats (male: 0/49; 3/50; 4/50; female: 8/50; 15/49; 18/50).

At no site were the incidences of neoplastic lesions in dosed mice considered to be compound related. Cytoplasmic vacuolization (fatty metamorphosis) of the liver was observed at an increased incidence in high dose female mice (0/49; 1/49; 8/49).

Genetic Toxicology: Diphenhydramine hydrochloride was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in either the presence or absence of exogenous metabolic activation. Exposure to this chemical did not induce trifluorothymidine (Tft) resistance in mouse L5178Y lymphoma cells with or without metabolic activation. In cytogenetic tests with cultured CHO cells, diphenhydramine hydrochloride induced chromosomal aberrations in the absence, but not the presence, of exogenous metabolic activation (S9); no induction of sister chromatid exchanges (SCEs) was observed in these cells with or without S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of diphenhydramine hydrochloride for male F344/N rats, based on marginally increased incidences of uncommon brain neoplasms (astrocytomas or gliomas) and of alveolar/bronchiolar neoplasms. There was *equivocal evidence of carcinogenic activity* for female F344/N rats, based on a marginal increase in the incidence of pituitary gland adenomas. There was *no evidence of carcinogenic activity* for male or female $B6C3F_1$ mice fed diets containing 156 or 313 ppm diphenhydramine hydrochloride.

Synonyms: 2-diphenylmethoxy-*N,N*-dimethylethanamine hydrochloride; 2-(benzhydryloxy)-*N,N*-dimethylethylamine hydrochloride; β-dimethylaminoethyl benzhydryl ether hydrochloride; benzhydramine hydrochloride

Trade Names: Alleran; Benadryl

Report Date: September 1989

TR-356 Toxicology and Carcinogenesis Studies of Furosemide (CAS No. 54-31-9) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Furosemide is a diuretic used in human and veterinary medicine. Toxicology and carcinogenesis studies were conducted by feeding diets containing furosemide (99% pure, USP grade) to groups of F344/N rats and $B6C3F_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: Dietary concentrations of furosemide used in the 14-day studies for rats and mice ranged up to 46,000 ppm. Two of five male and 3/5 female rats that received 46,000 ppm furosemide died before the end of the studies. Rats that received 15,300 or 46,000 ppm lost weight over the course of the studies. The final mean body weights of rats that received 1,700 or 5,100 ppm were 12% or 23% lower than that of controls for males and 8% or 16% lower for females. Nephrosis was dose related in rats. All five male and 1/5 female mice that received 46,000 ppm furosemide died before the end of the 14-day studies. Male mice that received 15,300 ppm and female mice that received 46,000 ppm lost weight. The final mean body weights of male mice that received 1,700 or 5,100 ppm were 16% or 14% lower than that of controls. The final mean body weight of females that received 15,300 ppm was 13% lower than that of controls. Slight dilatation of the renal cortical tubules and/or nephrosis were dose related in mice.

Dietary concentrations of furosemide used in the 13-week studies were 0 and 625-10,000 ppm for male rats and 0 and 938-15,000 ppm for female rats and male mice. Concentrations for female mice were 0 and 1,250-20,000 ppm. None of the rats died before the end of the studies. The final mean body weights of male rats that received 2,500, 5,000, or 10,000 ppm furosemide were 11%, 22%, or 44% lower than that of controls. The final mean body weights of female rats that received 3,750, 7,500, or 15,000 ppm were 18%, 26%, or 35% lower than that of controls. Minimal-to-mild nephrosis occurred in the two highest dose groups of male and female rats. Mineralization of minimal to mild severity was observed at the renal corticomedullary junction in dosed male rats receiving 625 ppm or more; the severity and incidence of the mineralization increased with increased dose. No compound-related deaths occurred in mice. The final mean body weights of male mice that received 3,750, 7,500, or 15,000 were 12%, 22%, or 17% lower than that of controls. Final mean body weights of dosed and control female mice were comparable. Compound-related lesions in mice induced minimal-to-mild nephrosis.

Because of the lower body weights and the kidney lesions in the 13-week studies, doses selected for the 2-year studies were 0, 350, or 700 ppm furosemide in the diet for groups of 50 F344/N rats of each sex. Groups of 50 $B6C3F_1$ mice of each sex were fed diets containing 0, 700, or 1,400 ppm furosemide for 104 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed and control rats were comparable throughout the studies. No significant differences in survival were observed between any groups of rats of either sex (final survival – male: control, 17/50; low dose, 17/50; high dose, 20/50; female: 35/50; 31/50; 34/50). The final survival of all groups of male rats was low, reflecting the large number of moribund animals killed after week 91. Survival at week 90 was 35/50, 28/50, and 34/50. Mean body weights of high dose male mice were up to 17% lower than those of controls, and mean body weights of low dose male mice were about 5%-10% lower than those of controls after week 31. Mean body weights of high dose female mice were up to 22% lower than those of controls. Mean body weights of low dose female mice were 5%-13% lower than those of controls

after week 82. The survival of the high dose group of female mice was significantly lower than that of controls after week 99 (final survival – male: 31/50; 24/50; 26/50; female: 36/50; 29/50; 18/50). Feed consumption by dosed rats was similar to that by controls. The estimated average amount of furosemide consumed per day was approximately 14-16 or 29-31 mg/kg for low dose or high dose rats. Feed consumption by dosed mice was approximately 5%-7% greater than that by controls. The average amount of furosemide consumed per day was approximately 91-99 or 191-214 mg/kg for low dose or high dose mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nephropathy occurred at similar incidences in all groups of rats, but the severity was greater in dosed male rats. Tubular cell hyperplasia was observed in 4/50 control, 2/50 low dose, and 4/50 high dose male rats. Tubular cell adenomas of the kidney occurred in 1/50 control, 3/50 low dose, and 1/50 high dose male rats. Tubular cell adenocarcinomas were seen in a fourth low dose male rat and in a second high dose male rat (adenomas or adenocarcinomas, combined: control, 1/50; low dose, 4/50; high dose, 2/50). The historical incidence of renal tubular cell adenomas or adenocarcinomas (combined) in untreated male F344/N rats is 9/1,928 (0.5%), and the highest incidence observed in controls is 3/50.

Malignant meningiomas of the brain occurred in 3/50 low dose male rats; none was observed in other groups. The historical incidence of meningiomas in untreated male F344/N rats is 2/1,928 (0.1%).

C-Cell adenomas of the thyroid gland in female rats occurred with a positive trend; the incidence in the high dose group was not statistically greater than that in the controls (4/50; 6/50; 11/50). A C-cell carcinoma occurred in another low dose female rat. The incidence of adenomas of the anterior pituitary gland in low dose male rats was marginally greater than that in controls (4/50; 11/50; 8/50). Neither of these marginal increases was considered to be chemically related.

Malignant mixed tumors (adenocarcinoma, type C) of the mammary gland occurred in dosed female mice (0/50; 1/50; 5/48). One mammary gland acinar cell carcinoma occurred in a second low dose female mouse. The historical incidence of all malignant mammary gland neoplasms in untreated female $B6C3F_1$ mice is 40/2,040 (2%).

Compound-related nonneoplastic lesions of the kidney in mice included nephropathy and dilatation of the renal pelvis for males and females and tubular cysts, suppurative inflammation, and epithelial hyperplasia of the renal pelvis for males. Kidney lesions may have contributed to the low survival of high dose female mice.

Mucosal epithelial hyperplasia and submucosal chronic focal inflammation of the urinary bladder were observed at increased incidences in dosed male mice. Suppurative inflammation of the prostate was observed at an increased incidence in high dose male mice. Fighting may have contributed to urogenital lesions in male mice. Suppurative inflammation of the ovary or uterus was observed at an increased incidence in high dose female mice. Hematopoiesis was observed at increased incidences in the spleen and liver of dosed male and high dose female mice and in the adrenal cortex of high dose female mice.

Genetic Toxicology: Furosemide was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without exogenous metabolic activation. In the mouse lymphoma assay for trifluorothymidine (Tft) resistance, furosemide produced an equivocal response in the absence of metabolic activation and a positive response in the presence of activation. Furosemide induced sister chromatid exchanges and chromosomal aberrations in CHO cells in both the presence and absence of exogenous metabolic activation.

Audit: The data, documents, and pathology materials from the 2-year studies of furosemide have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusion: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of furosemide for male F344/N rats, as shown by marginal increases in uncommon tubular cell neoplasms of the kidney and meningiomas of the brain. There was *no evidence of carcinogenic activity* of furosemide for female F344/N rats fed diets containing 350 or 700 ppm furosemide for 2 years. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice fed diets containing 700 or 1,400 ppm furosemide for 2 years. There was *some evidence of carcinogenic activity* of furosemide for female mice, as shown by an increase in malignant tumors of the mammary gland.

Nephropathy was more severe in the kidney of male rats and of male and female mice fed diets containing furosemide than in controls.

Synonyms: 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid; frusemide; fursemide

Trade Names: Aisemide; Aluzine; Beronald; Desdemin; Diural; Dryptal; Errolon; Frusemin; Fulsix; Fuluvamide; Furosemide "Mita"; Katlex; Lasilix; Lasix; Lowpstron; Rosemide; Transit; Urosemide

Report Date: May 1989

TR-357 Toxicology and Carcinogenesis Studies of Hydrochlorothiazide (CAS No. 58-93-5) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Hydrochlorothiazide is a diuretic active at the distal convoluted tubule and collecting duct. Toxicology and carcinogenesis studies were conducted by feeding diets containing hydrochlorothiazide (USP grade, greater than 98% pure) to groups of F344/N rats and $B6C3F_1$ mice of each sex for 15 days, 13 weeks, 1 year, or 2 years. Additional studies were performed to evaluate teratologic effects in CD® rats and CD®-1 mice. Genetic toxicology studies were performed with Salmonella, Chinese hamster ovary (CHO) cells, mouse lymphoma cells, and Drosophila.

Fifteen-Day and Thirteen-Week Studies: All rats and mice lived to the end of the 15-day studies (dietary concentrations of 0 and 3,125-50,000 ppm). The final mean body weights of all dosed rat groups were 5%-11% lower than those of controls. The final mean body weights of the groups of male mice that received 6,250-50,000 ppm were 10%-14% lower than that of controls. The final mean body weights of dosed and control female mice were similar. Calculi were seen in the urinary bladder of 2/5 male and 2/5 female mice at 50,000 ppm and in 1/5 male and 1/5 female mice at 25,000 ppm.

All rats lived to the end of the first 13-week studies (dietary concentrations of 0 and 3,125-50,000 ppm). Final body weights of dosed rats were 7%-16% lower than those of controls. Mineralization in the kidney was observed in all dosed rats and because of this, additional 13-week studies in rats were conducted at lower dietary concentrations. All rats lived to the end of the second 13-week studies (dietary concentrations of 0 and 250-4,000 ppm). The final mean body weights of all dosed rat groups were 5%-10% lower than those of controls. Renal mineralization was dose related and judged to be minimal to mild at the lowest dose.

In the 13-week studies in mice, 7/10 males and 1/10 females that received 50,000 ppm hydrochlorothiazide died. The final mean body weights of mice that received 50,000 ppm were 11% lower than those of controls for males and females. Calculi were seen in the urinary bladder of mice that received hydrochlorothiazide at 12,500 ppm and above. Nephrosis occurred with dose-related incidences in mice receiving 12,500 ppm and above.

Based on these results, 2-year studies were conducted by feeding diets containing 0, 250, 500, or 2,000 ppm hydrochlorothiazide to groups of 50 male and 50 female rats for 105-106 weeks. Diets containing 0, 2,500, or 5,000 ppm hydrochlorothiazide were fed to groups of 50 male and 50 female mice for 103-104 weeks. Ten additional rats per sex and dose group were placed on study and killed at 1 year for blood-clotting studies and histopathologic examination.

Effects in the One-Year Studies: One of 10 female rats in the 1-year study group that received 2,000 ppm died with internal hemorrhage. In addition, evidence of hemorrhage was found in 11 of the 16 dosed female rats that died during the first year of the 2-year study. Hematologic analyses revealed no compound-related effects; however, activated partial thromboplastin times (APTTs) were highly variable and were lengthened in some dosed male rats. No effects on APTTs were seen for females, and no effects on prothrombin times or on the fibrinogen content of plasma were observed for dosed male or female rats. Nephropathy occurred in dosed and control rats, and the severity was judged to be greater in dosed male and high dose female rats. Increased incidences of mild focal renal mineralization were also seen in mid and high dose male rats and dosed female rats.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were 8%-25% lower than those of controls. Mean body weights of dosed and control mice were similar throughout the studies. No significant differences in survival were observed between rats or mice of either sex (rats – male: control, 18/50; low dose, 16/50; mid dose, 9/50; high dose, 11/50; female: 31/50; 26/50; 30/50; 27/50; mice – male: control, 43/50; low dose, 42/50; high dose, 43/50; female: 38/50; 40/50; 35/50). Survival of all groups of male rats was low because a large number of animals were killed in a moribund condition late in the study. The average daily feed consumption by dosed rats was 89%-94% that by controls. The average amount of hydrochlorothiazide consumed per day was approximately 11, 23, or 89 mg/kg for low, mid, or high dose rats. The average daily feed consumption by dosed mice was 100%-105% that by controls. The average amount of hydrochlorothiazide consumed per day was approximately 280 or 575 mg/kg for low dose or high dose mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nephropathy occurred in nearly all male and female rats, but the severity of this disease was greater in dosed rats, as evidenced by increases in renal cysts and epithelial hyperplasia of the renal pelvis in dosed rats shown in the following table (see page 4 of the Technical Report). Mineralization was observed at increased incidences in dosed male and dosed female rats.

Changes associated with or secondary to renal injury were increased in dosed rats. These lesions included parathyroid hyperplasia, fibrous osteodystrophy of bone, and mineralization of multiple organs.

Adenomas or carcinomas (combined) of the Zymbal gland in male rats occurred in 1/50 control, 1/49 low dose, 2/50 mid dose, and 4/50 high dose animals. The historical incidence of Zymbal gland neoplasms in untreated F344/N rats is 19/1,936 (1.0%), and the highest observed control group incidence is 4/50. This marginal increase was not considered to be chemically related.

The incidences of fibroadenomas of the mammary gland were decreased in dosed female rats (30/50; 12/50; 11/49; 5/50).

The incidence of hepatocellular neoplasms was increased in high dose male mice (adenomas or carcinomas, combined: control, 7/48; low dose, 10/49; high dose, 21/50). The historical incidence of hepatocellular adenomas or carcinomas (combined) is 609/2,032 (30%) in untreated controls.

Teratology: Hydrochlorothiazide produced no teratologic effects in the offspring of CD® rats or CD®-1 mice after gavage administration to pregnant females on day 6 through day 15 of gestation.

Genetic Toxicology: In the absence of exogenous metabolic activation, hydrochlorothiazide produced an equivocal increase in revertant colonies in *Salmonella typhimurium* strain TA98; no increase was observed in strains TA100, TA1535, or TA1537 with or without activation. Hydrochlorothiazide induced an increase in trifluorothymidine (Tft)-resistant cells in a mouse lymphoma L5178Y/TK$^{+/-}$ assay without exogenous metabolic activation; this assay was not performed with activation. In cultured CHO cells, hydrochlorothiazide induced sister chromatid exchanges (SCEs) in the presence and absence of exogenous metabolic activation but did not induce chromosomal aberrations. Hydrochlorothiazide did not increase the frequency of sex-

linked recessive lethal mutations when administered by feeding or injection to adult male *Drosophila melanogaster*.

Audit: The data, documents, and pathology materials from the 2-year studies of hydrochlorothiazide have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* of hydrochlorothiazide for male or female F344/N rats given feed containing 250, 500, or 2,000 ppm hydrochlorothiazide. There was *equivocal evidence of carcinogenic activity* of hydrochlorothiazide for male $B6C3F_1$ mice, based on increased incidences of hepatocellular neoplasms. There was *no evidence of carcinogenic activity* for female $B6C3F_1$ mice given diets containing 2,500 or 5,000 ppm hydrochlorothiazide.

Chronic renal disease was more severe in rats administered hydrochlorothiazide, and increased incidences of secondary lesions (parathyroid hyperplasia, fibrous osteodystrophy, and mineralization in multiple organs) occurred in dosed rats.

Synonym: 6-chloro-3,4-dihydro-*2H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide

Trade Names: Aquarius; Bremil; Chlorzide; Cidrex; Dichlorosal; Dichlotride; Diclotride; Direma; Disalunil; Esidrix; Fluvin; Hidroronol; Hydril; Hydro-Aquil; Hydro-Diuril; Hydrosaluric; Hydrothide; Hypothiazide; Ivaugan; Jen-Diril; Maschitt; Nefrix; Neo-Codema; Neoflumen; Oretic; Panurin; Ro-Hydrazide; Thiaretic; Thiuretic; Urodiazin; Vetidrex

Report Date: July 1989

TR-358 Toxicology and Carcinogenesis Studies of Ochratoxin A (CAS No. 303-47-9) in F344/N Rats (Gavage Studies)

Ochratoxin A is a naturally occurring fungal toxin that is a contaminant in corn, peanuts, storage grains, cottonseed, meats, dried fish, and nuts. Toxicology and carcinogenesis studies were conducted by administering ochratoxin A (98% pure) in corn oil by gavage to groups of F344/N rats of each sex for 16 days, 13 weeks, 9 months, 15 months, or 2 years. Only rats were studied because ochratoxin A has been shown to be carcinogenic in mice. Genetic toxicology tests were performed with bacterial and mammalian cells. Urinalysis, hematologic and serum chemical analyses, and bone marrow cellularity determinations were conducted at 9, 15, and 24 months in the 2-year studies.

Sixteen-Day and Thirteen-Week Studies: Rats were administered 0, 1, 4, or 16 mg/kg ochratoxin A in corn oil by gavage 5 days per week for a total of 12 doses over 16 days. All rats that received 16 mg/kg ochratoxin A died within 6 days. Rats that received 4 mg/kg lost weight. Compound-related lesions in rats included bone marrow hyperplasia, thymic atrophy, necrosis and hyperplasia of the forestomach epithelium, renal tubular cell degenerative and regenerative changes (nephropathy), and adrenal gland hemorrhage. Renal tubular changes were most severe in animals that received 4 mg/kg. Rats that received 16 mg/kg had less severe renal lesions than those at 4 mg/kg, perhaps because the acute toxicity and early death did not allow sufficient time for full development of lesions.

No compound-related deaths occurred in the 13-week studies (doses were 0 and 0.0625 to 1 mg/kg). The final mean body weight of rats that received 0.25, 0.5, or 1 mg/kg was 7%, 11%, or 19% lower than that of vehicle controls for males and 3%, 4%, or 9% lower for females. Compound-related lesions in the kidney were characterized as degeneration and regeneration of the epithelium of the proximal convoluted tubules with individual cell necrosis of moderate severity (see page 3 of the Technical Report).

Karyomegaly of tubular epithelial cells was widespread but most pronounced in the straight portion of the tubules just above the corticomedullary junction. Karyomegaly was present in all dosed groups, and the severity increased as the dose increased. At lower doses, atrophy of the straight portions of the tubules at the corticomedullary junction and in the medulla was observed.

Based on mortality and on the presence and severity of renal lesions, groups of 80 rats per sex and dose group were administered 0, 21, 70, or 210 μg/kg ochratoxin A in corn oil by gavage 5 days per week for up to 2 years. Groups of 15 rats per sex and dose were killed at 9 or at 15 months and the remaining animals at 2 years.

Nine-Month and Fifteen-Month Studies: Administration of ochratoxin A by gavage for 9 months or 15 months to F344/N rats was associated with increased incidences of renal tubular cell neoplasms in males and hyperplasia, degeneration, and karyomegaly of renal tubular epithelial cells in both males and females (see page 4 of the Technical Report).

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose rats were generally 4%-7% lower than those of vehicle controls. No significant differences in survival were observed between any groups of female rats (vehicle control, 32/50; low dose, 23/51; mid dose, 35/50; high dose, 34/50). Survival was decreased after 77 weeks in high dose male rats and after 96 weeks in low and mid dose male rats (39/50; 26/51; 26/51; 23/50).

Clinical Pathology: Minor differences were observed for hematologic values between dosed and vehicle control animals, but these were not considered to be of biologic significance. Results of serum chemistry analysis were not clearly compound related. Ochratoxin A-dosed animals had slight increases compared with vehicle controls in urine volume and decreases in urine specific gravity in concentration tests, suggesting that exposure resulted in mild to moderate decreases in the ability to concentrate urine.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: A spectrum of degenerative and proliferative changes occurred in the kidney of male and female rats given ochratoxin A for 2 years. Degeneration of the renal tubular epithelium with formation of tubular cysts, proliferation of the tubular epithelium, and karyomegaly of the nuclei of tubular epithelial cells occurred at increased incidences in dosed rats (see page 5 of the Technical Report). Hyperplasia of the renal tubular epithelium and renal tubular adenomas and carcinomas also occurred at increased incidences in the dosed rats; the tumors were frequently multiple within a single kidney or were bilateral, and many metastasized to other organs.

The incidence of fibroadenomas of the mammary gland in high dose female rats was significantly greater than that in vehicle controls (vehicle control, 17/50; low dose, 23/51; mid dose, 22/50; high dose, 28/50). Multiple fibroadenomas of the mammary gland were observed at an increased incidence in high dose female rats (4/50; 4/51; 5/50; 14/50). One mammary gland adenoma was seen in a mid dose female, and two mammary gland adenocarcinomas were seen in each dosed group; one adenocarcinoma was seen in the vehicle control group.

An adenoma of the pars intermedia of the pituitary gland was observed in one mid dose female rat, and a carcinoma was observed in a second mid dose female rat. Squamous cell papillomas of the tongue were seen in two low dose and two mid dose male rats. Neither the pituitary neoplasms nor the papillomas of the tongue were considered related to ochratoxin A exposure.

Genetic Toxicology: Ochratoxin A was not mutagenic in four strains of *Salmonella typhimurium* (TA97, TA98, TA100, or TA1535) when tested both with and without exogenous metabolic activation. In cultured Chinese hamster ovary (CHO) cells, ochratoxin A induced sister chromatid exchanges (SCEs) in the presence, but not the absence, of metabolic activation; it did not significantly increase the number of chromosomal aberrations in these cells.

Audit: The data, documents, and pathology materials from the 2-year studies of ochratoxin A have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of ochratoxin A for male F344/N rats as shown by substantially increased incidences of uncommon tubular cell adenomas and of tubular cell carcinomas of the kidney. There was *clear evidence of carcinogenic activity* for female F344/N rats shown by increased incidences of uncommon tubular cell adenomas and of tubular cell carcinomas of the kidney and by increased incidences and multiplicity of fibroadenomas of the mammary gland.

Ochratoxin A administration also caused nonneoplastic renal changes including tubular cell hyperplasia, tubular cell proliferation, cytoplasmic alteration, karyomegaly, and degeneration of the renal tubular epithelium.

Synonym: (*R*)-*N*-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)-carbonyl](-L-)phenylalanine

Report Date: May 1989

TR-359 Toxicology and Carcinogenesis Studies of 8-Methoxypsoralen (CAS No. 298-81-7) in F344/N Rats (Gavage Studies)

Oral administration of 8-methoxypsoralen followed by exposure to longwave ultraviolet light (primarily ultraviolet A, 320-400 nm) is used in the treatment of vitiligo and psoriasis. 8-Methoxypsoralen also occurs naturally in a variety of vegetables. Toxicology and carcinogenesis studies of 8-methoxypsoralen without ultraviolet A were conducted by administering USP-grade 8-methoxypsoralen (99% pure) in corn oil by gavage to groups of F344/N rats once or for 16 days, 13 weeks, or 2 years. In vitro genetic toxicology tests were performed with bacteria and mammalian cells.

Single-Administration, Sixteen-Day, and Thirteen-Week Studies: In the single-administration studies, the chemical was administered at doses of 0 and 63-1,000 mg/kg. Four of five male rats and 5/5 female rats that received 1,000 mg/kg 8-methoxypsoralen died within 2 days.

In the 16-day studies, the chemical was administered at doses of 0 and 50-800 mg/kg. All rats receiving 800 mg/kg died within 5 days, and one male and one female at 400 mg/kg and one female at 200 mg/kg also died before the end of the studies. The final mean body weights of animals at 200 or 400 mg/kg were 14% or 30% lower than those of vehicle controls. No compound-related effects were observed at necropsy.

In the 13-week studies, the chemical was administered at doses of 0 and 25-400 mg/kg. Six of 10 male rats and 8/10 female rats that received 400 mg/kg died before the end of the studies. The final mean body weight of male rats that received 100, 200, or 400 mg/kg was 12%, 22%, or 45% lower than that of vehicle controls. The final mean body weight of female rats that received 200 or 400 mg/kg was 15% or 35% lower than that of vehicle controls. The liver weight to body weight ratios for all dosed groups of rats except the lowest (25 mg/kg) were greater than those for vehicle controls. Compound-related effects included fatty change in the liver in males and females and atrophy of the testis, seminal vesicles, and prostate.

Based on these results, 2-year studies were conducted by administering 0, 37.5 or 75 mg/kg 8-methoxypsoralen in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 F344/N rats of each sex.

Body Weight and Survival in the Two-Year Studies: The mean body weights of dosed male rats were generally 3%-14% lower than those of vehicle controls, and the mean body weights of high dose female rats were 5%-17% lower. The survival of both the low and the high dose groups of male rats was lower than that of the vehicle controls (male: vehicle control, 30/50; low dose, 16/50; high dose, 16/50; female: 39/50; 33/50; 36/50), likely because of kidney toxicity and neoplasia.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Mineralization of the renal papilla was observed in high dose male rats (vehicle control, 0/50; low dose, 0/50; high dose, 31/49). The severity of nephropathy was increased in dosed male rats. Focal hyperplasia of renal tubular cells was observed in dosed male rats (0/50; 8/50; 8/49). The incidences of tubular cell adenomas (1/50; 11/50; 8/49), adenocarcinomas (0/50; 1/50; 3/49), and adenomas or adenocarcinomas (combined) (1/50; 12/50; 11/49) were increased in dosed male rats. Hyperplasia of the parathyroid glands (2/49; 22/47; 18/48) and fibrous osteodystrophy (2/50; 10/50; 12/49) in male rats were secondary to chronic nephropathy.

The incidences of carcinomas or squamous cell carcinomas (combined) of the Zymbal gland were increased in dosed male rats (1/50; 7/50; 4/49). The mean historical incidence for carcinomas or squamous cell carcinomas (combined) in corn oil vehicle control male F344/N rats is 0.8% (16/1,949); the highest incidence in any one group is 4% (2/49).

Fibromas of the subcutaneous tissue in male rats occurred with a positive trend (1/50; 5/50; 7/49). An additional high dose male had a sarcoma. The mean historical incidence of fibromas or fibrosarcomas (combined) of subcutaneous tissue in corn oil vehicle control male F344/N rats is 9% (171/1,949).

Alveolar/bronchiolar adenomas occurred with a positive trend in male rats (4/50; 9/50; 9/49).
The mean historical incidence of alveolar/bronchiolar neoplasms in corn oil vehicle control male F344/N rats is 3% (68/1,944); the highest incidence is 10% (5/50).

Chronic inflammation, ulcers, and epithelial hyperplasia of the forestomach were observed at increased incidences in dosed male rats (chronic inflammation: 1/50; 6/50; 5/49; ulcers: 5/50; 13/50; 11/49; epithelial hyperplasia: 4/50; 19/50; 20/49). Squamous cell papillomas were observed in two low dose male rats.

Squamous cell papillomas were observed in the palate or tongue of one low dose and three high dose female rats; none were observed in vehicle controls. These papillomas were not considered to be related to chemical administration.

Diffuse hypertrophy of the thyroid gland was observed at increased incidences in dosed male rats (2/50; 31/50; 39/49).

Genetic Toxicology: 8-Methoxypsoralen was mutagenic in *Salmonella typhimurium* strain TA104 in the presence and absence of activation and in strains TA98, TA100, and TA102 when tests were conducted with exogenous metabolic activation; 8-methoxypsoralen was not mutagenic with or without activation in strain TA1535. Treatment with 8-methoxypsoralen induced both sister chromatid exchanges (SCEs) and chromosomal aberrations in Chinese hamster ovary (CHO) cells in the absence of exogenous metabolic activation; in the presence of activation, in the presence of activation, induction of SCEs occurred, but no significant increases in chromosomal aberrations was observed.

Audit: The data, documents, and pathology materials from the 2-year studies of 8-methoxypsoralen have been audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of 8-methoxypsoralen (without ultraviolet radiation) for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney and carcinomas of the Zymbal gland. Subcutaneous tissue fibromas and alveolar/bronchiolar adenomas of the lung in male F344/N rats may have been related to chemical administration. Dose-related nonneoplastic lesions in male F344/N rats included increased severity of nephropathy and mineralization of the kidney and forestomach lesions. There was *no evidence of carcinogenic activity* of 8-methoxypsoralen for female F344/N rats given the chemical at 37.5 or 75 mg/kg per day for 2 years.

Synonyms: 9-methoxy-7*H*-furo[3,2-*g*]benzopyran-7-one; 6-hydroxy-7-methoxy-5-benzofuranacrylic acid 6-lactone; 8-MP; 8-MOP; 8-methoxy-(furano-3',2':6,7-coumarin); 8-methoxy-4',5':6,7-furocoumarin; 9-methoxypsoralen; 8-methoxypsoralene; methoxsalen; oxypsoralen

Trade Names: Ammoidin; Meladinin (VAN); Meladinine; Meladoxen; Meloxine; Methoxa-Dome; Mopsoralen; Oxsoralen; Soloxsalen; Trioxun; Xanthotoxin; Xanthotoxine

Report Date: July 1989

TR-360 Toxicology and Carcinogenesis Studies of *N,N*-Dimethylaniline (CAS No. 121-69-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

N,N-Dimethylaniline is used as a chemical intermediate in the synthesis of dyestuffs. Toxicology and carcinogenesis studies were conducted by administering *N,N*-dimethylaniline (greater than 98% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 2 weeks, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells.

Two-Week and Thirteen-Week Studies: In the 2-week studies, doses were 94-1,500 mg/kg; deaths of rats and mice were observed in groups given doses of 750 or 1,500 mg/kg. The final mean body weights of male rats that received 375 or 750 mg/kg were 15% or 47% lower than that of vehicle controls; final mean body weights of other groups of rats and mice were similar to those of vehicle controls. Compound-related clinical signs observed included cyanosis in rats and lethargy and tremors in rats and mice. Splenomegaly occurred in nearly all dosed groups of rats and mice, and the incidences were dose related.

In the 13-week studies, doses were 32-500 mg/kg; no compound-related deaths occurred. The final mean body weights of male rats that received 250 or 500 mg/kg were 15% or 27% lower than that of vehicle controls. The final mean body weights of all groups of dosed female rats and male and female mice were within 12% of those of vehicle controls. Compound-related clinical signs included lethargy in rats and mice and cyanosis in rats. Splenomegaly was observed in all dosed groups of rats and mice; the severity was dose related. Compound-related extramedullary hematopoiesis and hemosiderosis occurred in the kidney or testis of dosed rats and liver and spleen of dosed rats and mice.

Two-year studies were conducted by administering 0, 3, or 30 mg/kg *N,N*-dimethylaniline in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 rats of each sex. The lower dose was selected to be one-tenth the higher dose to increase the likelihood that one dose would cause only a minimal nonneoplastic response. Groups of 50 mice of each sex were administered 0, 15, or 30 mg/kg on the same schedule.

Body Weight and Survival in the Two-Year Studies: Mean body weights of vehicle control and dosed rats and mice were similar throughout the studies. Survival rates of all respective groups were similar after 2 years, except for the lowered survival of vehicle control female rats (vehicle control, 21/50; low dose 32/50; high dose, 36/50). This may reflect the large number (24/50) of vehicle control female rats killed when observed to be in a moribund state. Final survival for other groups was as follows: male rats – 29/50; 32/50; 28/50; male mice – 34/50; 30/50; 34/50; female mice – 35/50; 39/50; 33/50.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: In these 2-year studies, the spleen was the expected site of chemical-related effects. Fatty metamorphosis and fibrosis in the spleen of high dose male rats were increased (fatty metamorphosis: vehicle control, 0/49; low dose, 1/49; high dose, 10/50; fibrosis: 5/49; 2/49; 22/50). Splenic hemosiderosis and hematopoiesis were present at an incidence greater than 85% in all groups of rats; however, the severity of the lesions was greater in dosed groups than in vehicle controls. Sarcomas of the spleen were seen in 3/50 high dose male rats, and an osteosarcoma was seen in another high dose male rat. One additional high dose male rat had a sarcoma of the thymus. Splenic sarcomas are uncommon in corn oil vehicle control male F344/N rats (NTP historical incidence 3/2,081, 0.1%), and thus, these neoplasms in high dose male rats (4/50, 8%) were considered to be chemically related.

Lower incidences of mononuclear cell leukemia (which apparently originates in the spleen) were seen in dosed male and female rats than in vehicle controls (male: 13/50; 4/50; 3/50; female: 11/50; 7/50; 0/50).

The incidence of squamous cell papillomas of the forestomach in high dose female mice was marginally greater than that in vehicle controls (2/50; 2/50; 8/50). No malignant forestomach neoplasms were observed.

Genetic Toxicology: *N,N*-Dimethylaniline was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of exogenous metabolic activation. In the mouse lymphoma assay, *N,N*-dimethylaniline produced a positive response with and without metabolic activation. In CHO cells, *N,N*-dimethylaniline induced both sister chromatid exchanges (SCEs) and chromosomal aberrations in the presence of exogenous metabolic activation. Without activation, an increase in chromosomal aberrations was observed, but no increase in SCEs occurred.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of *N,N*-dimethylaniline for male F344/N rats, as indicated by the increased incidences of sarcomas or osteosarcomas (combined) of the spleen. There was *no evidence of carcinogenic activity* of *N,N*-dimethylaniline for female F344/N rats given 3 or 30 mg/kg body weight by gavage for 2 years. There was *no evidence of carcinogenic activity* of *N,N*-dimethylaniline for male $B6C3F_1$ mice given 15 or 30 mg/kg body weight by gavage for 2 years. There was *equivocal evidence of carcinogenic activity* of *N,N*-dimethylaniline for female $B6C3F_1$ mice, as indicated by an increased incidence of squamous cell papillomas of the forestomach. Both rats and mice could have tolerated doses higher than those used in these studies.

There were decreased incidences of mononuclear cell leukemia in dosed male and high dose female rats. Compound-related splenic fibrosis, hemosiderosis, and fatty metamorphosis were increased in male rats.

Synonyms: dimethylaminobenzene; *N,N*-dimethylbenzeneamine; dimethylaniline; dimethylphenylamine; *N,N*-dimethylphenylamine

Report Date: October 1989

TR-361 Toxicology and Carcinogenesis Studies of Hexachloroethane (CAS No. 67-72-1) in F344/N Rats (Gavage Studies)

Hexachloroethane is used in organic synthesis as a retarding agent in fermentation, as a camphor substitute in nitrocellulose, in pyrotechnics and smoke devices, in explosives, and as a solvent. In previous long-term gavage studies with $B6C3F_1$ mice and Osbourne-Mendel rats (78 weeks of exposure followed by 12-34 weeks of observation), hexachloroethane caused increased incidences of hepatocellular carcinomas in mice. However, survival of low and high dose rats was reduced compared with that of vehicle controls, and the effects on rats were inconclusive. Therefore, additional toxicology and carcinogenesis studies were conducted in F344/N rats by administering hexachloroethane (approximately 99% pure) in corn oil by gavage to groups of males and females for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and in Chinese hamster ovary (CHO)

cells. Urinalysis was performed in conjunction with the 13-week studies.

Sixteen-Day Studies: In the 16-day studies (dose range, 187-3,000 mg/kg), all rats that received 1,500 or 3,000 mg/kg and 1/5 males and 2/5 females that received 750 mg/kg died before the end of the studies. Final mean body weights of rats that received 750 mg/kg were 25% lower than that of vehicle controls for males and 37% lower for females. Compound-related clinical signs seen at 750 mg/kg or more included dyspnea, ataxia, prostration, and excessive lacrimation. Other compound-related effects included hyaline droplet formation in the tubular epithelial cells in all dosed males and tubular cell regeneration and granular casts in the tubules at the corticomedullary junction in the kidney in males receiving 187 and 375 mg/kg.

Thirteen-Week Studies: In the 13-week studies (dose range, 47-750 mg/kg), 5/10 male rats and 2/10 female rats that received 750 mg/kg died before the end of the studies. The final mean body weight of male rats that received 750 mg/kg was 19% lower than that of vehicle controls. Compound-related clinical signs for both sexes included hyperactivity at doses of 94 mg/kg or higher and convulsions at doses of 375 or 750 mg/kg. The relative weights of liver, heart, and kidney were increased for exposed males and females. Kidney lesions were seen in all dosed male groups, and the severity increased with dose. Papillary necrosis and tubular cell necrosis and degeneration in the kidney and hemorrhagic necrosis in the urinary bladder were observed in the five male rats that received 750 mg/kg and died before the end of the studies; at all lower doses, hyaline droplets, tubular regeneration, and granular casts were present in the kidney. No chemical-related kidney lesions were observed in females. Foci of hepatocellular necrosis were observed in several male and female rats at doses of 188 mg/kg or higher.

Dose selection for the 2-year studies was based primarily on the lesions of the kidney in males and of the liver in females. Studies were conducted by administering hexachloroethane in corn oil by gavage at 0, 10, or 20 mg/kg body weight, 5 days per week, to groups of 50 male rats. Groups of 50 female rats were administered 0, 80, or 160 mg/kg on the same schedule.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose rats were slightly (5%-9%) lower than those of vehicle controls toward the end of the studies. No significant differences in survival were observed between any groups of rats (male: vehicle control, 31/50; 10 mg/kg, 29/50; 20 mg/kg, 26/50; female: vehicle control, 32/50; 80 mg/kg, 27/50; 160 mg/kg, 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Incidences of kidney mineralization (vehicle control, 2/50; low dose, 15/50; high dose, 32/50) and hyperplasia of the pelvic transitional epithelium (0/50; 7/50; 7/50) were increased in dosed male rats. Renal tubule hyperplasia was observed at an increased incidence in high dose male rats (2/50; 4/50; 11/50). These lesions have been described as characteristic of the hyaline droplet nephropathy that is associated with an accumulation of liver-generated $\alpha_2\mu$-globulin in the cytoplasm of tubular epithelial cells. The severity of nephropathy was increased in high dose male rats (moderate vs. mild), and the incidences and severity of nephropathy were increased in dosed females (22/50; 42/50; 45/50). The incidences of adenomas (1/50; 2/50; 4/50), carcinomas (0/50; 0/50; 3/50), and adenomas or carcinomas (combined) (1/50; 2/50; 7/50) of the renal tubule were also increased in the high dose male group. One of the carcinomas in the high dose group metastasized to the lung. No compound-related neoplasms were observed in females.

The incidence of pheochromocytomas of the adrenal gland in low dose male rats was significantly greater than that in vehicle controls (15/50; 28/50; 21/49), and the incidences for both dosed groups were greater than the mean historical control incidence (28% ± 11%).

Genetic Toxicology: Hexachloroethane was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with and without exogenous metabolic activation. In CHO cells, hexachloroethane did not induce chromosomal aberrations with or with out metabolic activation but did produce sister chromatid exchanges in the presence of exogenous metabolic activation.

Audit: The data, documents, and pathology materials from the 2-year studies of hexachloroethane have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of hexachloroethane for male F344/N rats, based on the increased incidences of renal neoplasms. The marginally increased incidences of pheochromocytomas of the adrenal gland may have been related to hexachloroethane administration to male rats. There was *no evidence of carcinogenic activity* of hexachloroethane for female F344/N rats administered 80 or 160 mg/kg by gavage for 103 weeks.

The severity of nephropathy and incidences of linear mineralization of the renal papillae and hyperplasia of the transitional epithelium of the renal pelvis were increased in dosed male rats. The incidences and severity of nephropathy were increased in dosed female rats.

Synonyms: carbon hexachloride; ethane hexachloride; hexachlorethane; hexachloroethylene; 1,1,1,2,2,2-hexachloroethane; perchloroethane

Trade Names: Avlothane; Distokal; Distopan; Distopin; Egitol; Falkitol; Fasciolin; Mottenhexe; Phenohep

Report Date: August 1989

Note: Hexachloroethane was previously tested in Osborne-Mendel rats and B6C3F$_1$ mice by gavage (See TR-68, reported 1978).

TR-362 Toxicology and Carcinogenesis Studies of 4-Vinyl-1-cyclohexene Diepoxide (CAS No. 106-87-6) in F344/N Rats and B6C3F$_1$ Mice (Dermal Studies)

4-Vinyl-1-cyclohexene diepoxide is used a chemical intermediate and as a reactive diluent for diepoxides and epoxy resins. Toxicology and carcinogenesis studies were conducted by administering 4-vinyl-1-cyclohexene diepoxide (97% pure) in acetone by dermal application to individually housed F344/N rats and B6C3F$_1$ mice for 14 days, 13 weeks, 15 months, and 2 years. Additional studies included evaluation of immune function after a 5-day dermal exposure and evaluation of the oral toxicity of 4-vinyl-1-cyclohexene diepoxide in 16-day and 13-week corn oil gavage studies. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day Dermal Studies: In the 14-day studies, all rats that received 924 mg/kg or higher (equivalent to 139 mg/rat or higher for males and 112 mg/rat of higher for females) died before the end of the studies. Final mean body weights were lower than those of vehicle controls in males receiving 68 mg/rat and in females receiving 57 mg/rat. Excoriations on the skin at the application site were observed in the groups receiving 57 mg/rat or more. Males receiving 139 mg/rat and females receiving 112 mg/rat had congestion and/or hypoplasia of the bone marrow; most had acute nephrosis. Skin lesions, including epidermal necrosis and ulceration, epidermal hyperplasia, and hyperkeratosis, were found in the 139 and 112 mg/rat group; similar lesions of lesser severity were seen in the two lowest dose groups of each sex.

All mice that received 1,787 mg/kg (equivalent to 43/mouse for males and 37 mg/mouse for females) and 3/5 male mice and 5/5 female mice that received 889 mg/kg (equivalent to 19-21 mg/mouse) died before the end of the 14-day dermal studies. Final mean body weights of exposed and vehicle control mice were generally similar. Lesions of the skin at the site of application were seen in 4/5 males and 4/5 females receiving 5 mg/mouse and all mice receiving 10 and 21 (males) or 19 (females) mg/mouse and included epidermal and sebaceous gland hyperplasia, hyperkeratosis, and ulceration.

Thirteen-Week Studies: In the 13-week dermal studies, all rats survived to the end of the studies (doses up to 60 mg/rat). The final mean body weights of the 60 mg/rat groups were 9%-14% lower than those of the vehicle controls. Compound-related clinical signs in the 60 mg/rat groups observed during the second half of the studies included redness, scabs, and ulceration at the application site and burrowing behavior after dermal application. Hyperplasia of the sebaceous glands and acanthosis (hyperplasia) and hyperkeratosis of the squamous epithelium were seen at the site of application.

In mice, no compound-related deaths occurred after applications of up to 10 mg/mouse in 13-week dermal studies, and final mean body weights of exposed and vehicle control mice were similar. Relative liver and kidney weights increased with dose. Compound-related lesions of the skin included sebaceous gland hyperplasia and acanthosis (hyperplasia) and hyperkeratosis of the stratified squamous epithelium at the site of application; ovarian atrophy was also considered to be compound related.

In the 13-week oral studies, the major target organ of toxicity in rats and mice was the forestomach, as indicated by hyperkeratosis and hyperplasia of the stratified squamous epithelium. In female mice, ovarian atrophy was seen in 4-vinyl-1-cyclohexene diepoxide-dosed groups.

Two-year studies were conducted by administering 4-vinyl-1-cyclohexene diepoxide in acetone by dermal application, 5 days per week for 105 weeks to groups of 60 rats of each sex at 0, 15, or 30 mg/animal. Groups of 60 mice of each sex were administered 0, 2.5, 5, or 10 mg/animal on the same schedule for 103 weeks. None of the doses selected had produced ulceration of skin in 13-week studies. Ten animals from each group were killed and examined during month 15 for toxicologic evaluation.

Immune Function Studies: The immunotoxic effects of 4-vinyl-1-cyclohexene diepoxide were studied in male B6C3F$_1$ mice after a 5-day dermal exposure at doses ranging from 2.5 to 10 mg/mouse per day. 4-Vinyl-1-cyclohexene diepoxide was immunosuppressive at 10 mg/mouse and, to a lesser extent, at 5 mg/mouse, as indicated by a decrease in peripheral lymphocytes and the in vitro lymphoproliferative response to phytohemagglutinin and concanavalin A in the high dose group and suppression of the antibody plaque-forming-cell response in the 5 and 10 mg/mouse groups.

Fifteen-Month Evaluation: Two of 10 male rats that received 30 mg had a squamous cell carcinoma of the skin at or adjacent to the site of application. Acanthosis was seen in exposed rats (mild severity at 30 mg/rat and minimal severity at 15 mg/rat); hyperkeratosis was observed for rats in the 30 mg/rat groups.

Compound-related nonneoplastic skin lesions in mice included acanthosis, hyperkeratosis, and sebaceous gland hyperplasia/hypertrophy. Squamous cell papillomas and carcinomas were seen in mice that received 5 or 10 mg/mouse; none was seen in vehicle control or low dose groups (papillomas—male: mid dose, 1/10; high dose, 2/10; female: 1/10; 1/10; carcinomas—male: 2/10; 8/10; female: 2/10; 5/10). One vehicle control and all exposed female mice had atrophy of the ovary. Hyperplasia of the ovarian surface epithelium was seen in 8/10 females receiving 5 mg/mouse and 9/9 females receiving 10 mg/mouse. Two of nine females receiving 10 mg/mouse had granulosa cell tumors of the ovary, and 1/9 females receiving 10 mg/mouse had an ovarian papillary cystadenoma.

Body Weights and Survival in the Two-Year Studies: In general, the body weights and survival were lower in mid and high dose groups than in vehicle controls. The survival was lower in exposed groups, primarily because of neoplasms (survival at week 105—male rats: vehicle control, 7/50; low dose, 8/50; high dose, 4/50; female rats: 27/50; 23/50; 15/50; male mice: vehicle control, 38/50; low

dose, 35/50; mid dose, 4/50; high dose, 0/50; female mice: 30/50; 31/50; 15/50; 0/50). All high dose male mice died by week 83; the 10 surviving high dose female mice were killed during week 85.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Acanthosis and sebaceous gland hypertrophy of skin from the scapula or back were observed at substantially increased incidences in exposed male and female rats. Squamous cell papillomas in male rats and squamous cell carcinomas in male and female rats were observed only in exposed rats (squamous cell carcinomas – male: vehicle control, 0/50; low dose, 33/50; high dose, 36/50; female: 0/50; 16/50; 34/50). The incidences of basal cell adenomas or carcinomas (combined) were increased (male: 0/50; 1/50; 6/50; female: 0/50; 3/50; 4/50).

For exposed mice, acanthosis, hyperkeratosis, and necrotizing inflammation of the skin were observed over the scapula or back. Squamous cell carcinomas were found only in exposed mice (male: vehicle control, 0/50; low dose, 14/50; mid dose, 39/50; high dose, 42/50; female: 0/50; 6/50; 37/50; 41/50).

Follicular atrophy and tubular hyperplasia of the ovary in female mice were increased (atrophy: 12/50; 43/49; 47/50; tubular hyperplasia: 5/50; 35/49; 38/49; 34/50). Mid and high dose females had benign or malignant granulosa cell tumors (0/50; 0/49; 7/49; 12/50) and benign mixed tumors (0/50; 0/49; 11/49; 6/50). The combined incidences of luteomas, granulosa cell tumors, benign mixed tumors, or malignant granulosa cell tumors in mid and high dose female mice were increased (1/50; 0/49; 17/49; 18/50).

The incidences of alveolar/bronchiolar adenomas or carcinomas (combined) in exposed female mice were marginally increased (4/50; 9/50; 11/50; 7/50).

Genetic Toxicology: 4-Vinyl-1-cyclohexene diepoxide was mutagenic in *S. typhimurium* strains TA98, TA100, and TA1535 with and without exogenous metabolic activation; the compound was equivocally mutagenic in strain TA1537 without S9 but gave a positive response in the presence of activation. 4-Vinyl-1-cyclohexene diepoxide induced resistance to trifluorothymidine in mouse L5178Y/TK cells without exogenous metabolic activation; it was not tested with activation. 4-Vinyl-1-cyclohexene diepoxide induced sister chromatid exchanges and chromosomal aberrations in CHO cells in the presence and absence of exogenous metabolic activation.

Conclusions: Under the conditions of these 2-year dermal studies, there was *clear evidence of carcinogenic activity* of 4-vinyl-1-cyclohexene diepoxide for male and female F344/N rats, as shown by squamous cell and basal cell neoplasms of the skin. There was *clear evidence of carcinogenic activity* of 4-vinyl-1-cyclohexene diepoxide for male and female $B6C3F_1$ mice, as shown by squamous cell carcinomas of the skin in males and squamous cell carcinomas of the skin and ovarian neoplasms in females; increased incidences of lung neoplasms in females may also have been related to chemical application.

Synonyms: 4-vinylcyclohexene diepoxide; 4-vinyl-1,2-cyclohexene diepoxide; 1-vinyl-3-cyclohexene diepoxide; 4-vinylcyclohexene dioxide; 1,2-epoxy-4-(epoxyethyl) cyclohexane; 1-epoxyethyl-3,4-epoxycyclohexane

Report Date: November 1989

TR-363 Toxicology and Carcinogenesis Studies of Bromoethane (Ethyl Bromide) (CAS No. 74-96-4) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

Bromoethane is an alkylating agent used primarily as a chemical intermediate in various organic syntheses. In toxicology and carcinogenesis studies, groups of F344/N rats and $B6C3F_1$ mice of each sex received whole-body exposure to bromoethane (greater than 98% pure) once for 4 hours or for 6 hours per day, 5 days per week, for 14 days, 14 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Single-Exposure, Fourteen-Day, and Fourteen-Week Studies: Single-exposure inhalation studies were conducted in rats and mice at target concentrations of 625, 1,250, 2,500, 5,000, or 10,000 ppm bromoethane. All rats exposed to 10,000 ppm bromoethane and 3/5 female rats exposed to 5,000 ppm died before the end of the single-exposure studies. All mice exposed to 5,000 or 10,000 ppm bromoethane and 2/5 female mice exposed to 1,250 ppm died before the end of the studies.

Fourteen-day inhalation studies were conducted in rats and mice at target concentrations of 0, 250, 500, 1,000, 2,000, or 4,000 ppm bromoethane. All rats and mice exposed to 2,000 or 4,000 ppm died before the end of the 14-day studies. Final mean body weights of exposed and control rats were similar.

Fourteen-week inhalation studies were conducted in rats and mice at target concentrations of 0, 100, 200, 400, 800, or 1,600 ppm bromoethane. Four of 10 male and 2/10 female rats exposed to 1,600 ppm died before the end of the 14-week studies. The final mean body weights of rats exposed to 1,600 ppm were lower than the initial mean body weights. Compound-related lesions observed in rats at 1,600 ppm, but not at lower concentrations, included minimal atrophy of the thigh muscle, minimal-to-moderate multifocal mineralization in the cerebellum of the brain, minimal-to-severe hemosiderosis of the spleen, marked atrophy of the testis, and minimal-to-mild atrophy of the uterus. The effects in the testis and uterus are probably due to chemical-related loss in body weight during the studies.

In mice, compound-related deaths included 3/10 male and 1/10 female mice exposed to 1,600 ppm, 1/9 males exposed to 800 ppm, and 1/10 males exposed to 400 ppm. The final mean body weights of male and female mice exposed to 1,600 ppm were about 15% lower than those of controls. Compound-related effects included atrophy of the uterus and involution of the ovary in females exposed to 1,600 ppm bromoethane.

Based on these results, 2-year studies were conducted by exposing groups of 49 or 50 rats or mice of each sex to bromoethane at 0, 100, 200, or 400 ppm, 6 hours per day, 5 days per week.

Body Weight and Survival in the Two-Year Studies: Mean body weights of exposed and control rats were generally similar throughout the studies. No significant differences in survival were observed between any groups of male rats (control, 17/49; 100 ppm, 26/50; 200 ppm, 27/50; 400 ppm, 21/50); survival of the 100-ppm group of female rats was greater than that of controls (19/50; 29/50; 24/49; 23/50), and the number of control and 400-ppm male rats and control female rats surviving to the end of the studies was low.

Mean body weights of the 400-ppm group of male mice were up to 9% lower than those of controls throughout the study. Mean body weights of the 400-ppm group of female mice were generally 6%-16% lower than those of controls after week 29. No differences in survival were observed between any group of male mice (35/50; 37/50; 30/50; 34/50). The survival of the 400-ppm group of female mice was lower than that of controls at the end of the study (36/50; 37/50; 37/49; 23/49).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The incidences of pheochromocytomas or malignant pheochromocytomas (combined) of the adrenal medulla were increased in exposed male rats (control, 8/40; 100 ppm, 23/45; 200 ppm, 18/46; 400 ppm, 21/46).

Granular cell neoplasms of the brain were seen in exposed male rats but not in controls (0/49; 3/50; 1/50; 1/50). A glioma, an astrocytoma, or an oligodendroglioma was seen in 3/50 male rats exposed to 100 ppm. Gliomas were not observed in control female rats, but they occurred in exposed female rats with a significant positive trend (0/50; 1/50; 1/48; 3/50). The historical incidence of granular cell tumors in male F344/N rat chamber controls at the study laboratory is 0/297. The incidences of gliomas in the exposed female groups were not significantly greater than that in the controls and were within the historical incidence range for glial cell neoplasms for untreated controls in NTP studies (mean: 23/1,969, 1%; range: 0/50-3/50), but they exceeded the historical incidence range for chamber controls at the study laboratory (mean: 1/297, 0.3%; range: 0/50-1/50).

Alveolar epithelial hyperplasia was increased in rats exposed to 400 ppm bromoethane (male: 3/48; 7/49; 7/48; 18/48; female: 5/50; 4/48; 5/47; 10/49). Alveolar/bronchiolar adenomas or carcinomas (combined) were seen in four male rats exposed to 200 ppm and in one exposed to 400 ppm. Alveolar/bronchiolar adenomas were observed in 3/49 female rats at 400 ppm but not at lower concentrations or in controls. The incidences in exposed male and female rats were not significantly greater than those in controls; however, the historical incidence in rat chamber controls for alveolar/bronchiolar adenomas or carcinomas (combined) at the study laboratory is 6/299 (2%) for males and 4/297 (1.3%) for females.

The incidences of epithelial hyperplasia and squamous metaplasia of the nasal cavity were increased in rats exposed to 400 ppm. The incidence of suppurative inflammation of the nasal cavity was increased in exposed male rats, and the incidences of suppurative inflammation of the larynx and metaplasia of the olfactory sensory epithelium were increased in exposed male and female rats. An adenoma of the nose was seen in one 400-ppm male rat and in one 400-ppm female mouse.

Suppurative inflammation and dilatation of the salivary gland ducts were observed at increased incidences in the 200- and 400-ppm groups of female rats. Animals were found to be positive for rat coronavirus/sialodacryoadenitis virus antibodies.

The incidence of mammary gland neoplasms was significantly lower in female rats at 400 ppm than in controls (18/50; 15/50; 10/48; 7/50).

Adenomas (0/50; 1/50; 1/47; 6/48), adenocarcinomas (0/50; 2/50; 3/47; 19/48), and squamous cell carcinomas (0/50; 1/50; 1/47; 3/48) of the uterus occurred in exposed female mice and not in control mice.

The incidence of alveolar/bronchiolar neoplasms was greater in male mice at 400 ppm than in controls (adenomas or carcinomas, combined: 7/50; 6/50; 12/50; 15/50). Acute/chronic inflammation of the lung was observed at increased incidences in female mice at 200 and 400 ppm.

Genetic Toxicology: Bromoethane, tested in a closed environment of a desiccator, was mutagenic in *S. typhimurium* strain TA100 with and without exogenous metabolic activation; it was not mutagenic in strain TA98. In cultured CHO cells, bromoethane induced sister chromatid exchanges (SCEs) but not chromosomal aberrations in both the presence and absence of exogenous metabolic activation.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity* of bromoethane for male F344/N rats, as indicated by increased incidences of pheochromocytomas of the adrenal gland; neoplasms of the brain and lung may also have been related to exposure to bromoethane. For female F344/N rats, there was *equivocal evidence of carcinogenic activity*, as indicated by marginally increased incidences of neoplasms of the brain and lung. For male $B6C3F_1$ mice, there was *equivocal evidence of carcinogenic activity*, based on marginally increased incidences of neoplasms of the lung. There was *clear evidence of carcinogenic activity* for female $B6C3F_1$ mice, as indicated by neoplasms of the uterus.

Synonyms: monobromoethane; bromic ether; hydrobromic ether

Report Date: October 1989

TR-364 Toxicology and Carcinogenesis Studies of Rhodamine 6G (C.I. Basic Red 1) (CAS No. 989-38-8) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Toxicology and carcinogenesis studies of rhodamine 6G were conducted because of potential human exposure

related to its use as a dye for natural and synthetic fibers and as a research chemical. These studies were conducted by administering rhodamine 6G (greater than 95% pure) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies (0, 310, 620, 1,250, 2,500, or 5,000 ppm), all five male and five female rats that received 5,000 ppm and 1/5 male rats that received 2,500 ppm died before the end of the studies; all mice lived to the end of the study. The final mean body weights of rats that received 2,500 ppm were lower than the initial weights. The final mean body weights of mice that received 2,500 or 5,000 ppm were 8% or 18% lower than that of controls for males and 2% or 8% lower for females.

In the 13-week studies, all rats lived to the end of the studies (dietary concentrations of 0 or 120-2,000 ppm). The final mean body weights of rats that received 500, 1,000 or 2,000 ppm were 12%, 13%, or 32% lower than that of controls for males and 4%, 8%, or 20% lower for females. Feed consumption by rats that received 2,000 ppm was somewhat lower than that by controls. Bone marrow atrophy was observed at increased incidences and severity in dosed rats. In the 13-week study (0 or 500-8,000 ppm), 1/10 male mice that received the highest concentration died before the end of the studies. The final mean body weights of mice that received 8,000 ppm were lower than the initial mean body weights. The final mean body weights of male mice that received 4,000 ppm and of female mice that received 2,000 or 4,000 ppm were 13%-19% lower than those of controls. Feed consumption was not related to dose. Minimal-to-moderate cytoplasmic vacuolization of hepatocytes was seen in 8/10 male mice that received 8,000 ppm.

Based on these results, dietary concentrations selected for the 2-year studies were 0, 120, or 250 ppm rhodamine 6G for rats, 0, 1,000, or 2,000 ppm for male mice, and 0, 500, 1,000 ppm for female mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were similar to those of controls throughout the studies. The average daily feed consumption by dosed rats was within 5% that by controls for all dosed groups. The average amount of rhodamine 6G consumed per day was approximately 5 mg/kg for low dose rats and 10 or 12 mg/kg for high dose male or female rats. Mean body weights of high dose male and dosed female mice were generally 5%-14% lower than those of controls. The average daily feed consumption by dosed mice was within 5% that by controls for all dosed groups. The average amount of rhodamine 6G consumed per day was approximately 210 or 440 mg/kg for low dose or high dose male mice and 125 or 250 mg/kg for low dose or high dose female mice. No significant differences in survival were observed between any groups of rats or mice (male rats: control, 22/50; low dose, 21/50; high dose, 27/50; female rats: 29/50; 30/50; 30/50; male mice: 36/50; 32/50; 38/50; female mice: 39/50; 35/50; 36/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: No chemically related nonneoplastic lesions in male or female rats and no chemically related neoplastic or nonneoplastic lesions in male or female mice were observed in these studies.

The incidences of keratoacanthomas of the skin was increased in high dose male rats (control, 1/50; low dose, 2/50; high dose, 8/50). The historical incidence of keratoacanthomas in untreated control male F344/N rats is 31/1,936 (1.6%; range, 0/50-7/49). Both fur and skin of rats in the dosed groups apparently were exposed to feed dust containing rhodamine 6G; the intensity of staining was proportional to the concentration of rhodamine 6G in feed. Because of the variable background incidence of keratoacanthomas in F344/N rats, the incidence of keratoacanthomas cannot be conclusively related to exposure to rhodamine 6G.

The incidences of pheochromocytomas (3/50; 3/50; 8/50) or malignant pheochromocytomas (combined: 3/50; 3/50; 10/50) of the adrenal gland were increased in high dose female rats. The historical incidence of adrenal medullary neoplasms in untreated control F344/N female rats is 99/1,968 (5%; range, 0/50-8/50). This marginal increase may be related to the administration of rhodamine 6G.

Genetic Toxicology: Rhodamine 6G was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with and without exogenous metabolic activation (S9). Rhodamine 6G gave a positive response in the absence of S9 in the mouse lymphoma assay for induction of trifluorothymidine (Tft) resistance in L5178Y cells; in the presence of S9, rhodamine 6G was negative. Rhodamine 6G induced sister chromatid exchanges (SCEs) and chromosomal aberrations in cultured CHO cells in the presence, but not the absence, of S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* for male F344/N rats administered rhodamine 6G, as indicated by a marginally increased incidence of integumentary keratoacanthomas. There was *equivocal evidence of carcinogenic activity* for female F344/N rats administered rhodamine 6G, as indicated by a marginal increase in pheochromocytomas or malignant pheochromocytomas (combined) of the adrenal gland. There was *no evidence of carcinogenic activity* for male B6C3F$_1$ mice administered 1,000 or 2,000 ppm rhodamine 6G in the diet. There was *no evidence of carcinogenic activity* for female B6C3F$_1$ mice administered 500 or 1,000 ppm rhodamine 6G in the diet.

There were no significant nonneoplastic lesions attributed to rhodamine 6G administration to male or female rats or male or female mice. Male and female rats might have been able to tolerate a higher concentration of rhodamine 6G in the feed.

Synonym: 2-[6-(ethylamino)-3-(ethylimino)2,7-dimethyl-3*H*-xanthen-9-yl] benzoic acid ethyl ester, monohydrochloride

Common Names: Basic Red 1; Basic Rhodamine Yellow; Basic Rhodaminic Yellow; Calcozine Red 6G; Calcozine

Rhodamine 6GX; C.I. Basic Red 1, Monohydrochloride; Elcozine Rhodamine 6GDN; Eljon Pink Toner; Fanal Pink GFK; Fanal Red 25532; Flexo Red 482; Heliostable Brilliant Pink B extra; Mitsui Rhodamine 6GCP; Nyco Liquid Red GF; Rhodamine 69DN Extra; Rhodamine F4G; Rhodamine F5G; Rhodamine F5G chloride; Rhodamine 6GB; Rhodamine 6GBN; Rhodamine 6GCP; Rhodamine 6GD; Rhodamine 4GD; Rhodamine GDN; Rhodamine 5GDN; Rhodamine 6 GDN; Rhodamine GDN Extra; Rhodamine 6GEx ethyl ester; Rhodamine 6G Extra; Rhodamine 6G Extra Base; Rhodamine 4GH; Rhodamine 6GH; Rhodamine 5GL; Rhodamine 6G lake; Rhodamine 6GX; Rhodamine J; Rhodamine 6JH; Rhodamine 7JH; Rhodamine Lake Red 6G; Rhodamine Y 20-7425; Rhodamine Zh; Rhodamine 6ZH-DN; Silosuper Pink B; Valley Fast Red 1308

Report Date: September 1989

TR-365 Toxicology and Carcinogenesis Studies of Pentaerythritol Tetranitrate (CAS No. 78-11-5) with 80% D-Lactose Monohydrate (PETN, NF) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Pentaerythritol tetranitrate (PETN, NF) is a drug used to prevent angina pectoris. PETN without a lactose stabilizer is used as an explosive. Toxicology and carcinogenesis studies were conducted by administering PETN, NF, to groups of F344/N rats and B6C3F$_1$ mice of each sex once by gavage or in feed for 14 days, 13 or 14 weeks, or 2 years. The PETN component was greater than 99% pure. Genetic toxicology studies were conducted with *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: All rats and mice lived to the end of the 14-day studies (dietary concentrations up to 50,000 ppm). Final mean body weights of dosed and control rats were comparable. The final mean body weight of female mice that received 50,000 ppm was 13% lower than that of controls. No clinical signs or toxic lesions were attributed to PETN, NF, administration.

All rats and mice lived to the end of the 13-week (mice) and 14-week (rats) studies (dietary concentrations up to 50,000 ppm). Final mean body weights of dosed and control rats and mice were similar, although weight gains of female rats at 25,000 and 50,000 ppm were less than that of controls. The nitrite level in urine of rats and methemoglobin levels in whole blood of rats and mice were not affected by administration of PETN, NF. An adenoma of the Zymbal gland was seen in a female rat that received 50,000 ppm. A hepatocellular adenoma was seen in a female mouse that received 50,000 ppm.

Based on these results and the NTP convention of limiting concentrations in 2-year feed studies to 5% of the diet, the 2-year studies were conducted by administering 0, 25,000 or 50,000 ppm PETN, NF, in feed for 104 weeks to groups of 50 male rats and for 103 weeks to groups of 49 or 50 mice of each sex. Groups of 50 female rats were given feed containing 0, 6,200, or 12,500 ppm PETN, NF, for 104 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male rats were 2%-9% lower than those of controls throughout the study; body weights of all groups of female rats were similar. No significant differences in survival were observed between any groups of rats of either sex (male: control, 23/50; low dose, 29/50; high dose, 29/50; female: 33/50; 33/50; 31/50). Mean body weights of dosed and control mice were similar. The survival of both groups of dosed male mice was significantly greater than that of the controls (26/49; 38/50; 38/50). No significant differences in survival were observed between any groups of female mice (38/50; 30/50; 38/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: No nonneoplastic lesions were attributed to PETN, NF, administration in rats or mice. Neoplasms of the Zymbal gland occurred in dosed male (control, 0/49; low dose, 3/45; high dose, 2/41) and dosed female (0/36; 1/37; 3/35) rats. The historical incidence of these neoplasms is 1% ± 2% in untreated males and 0.6% ± 1% in females.

At no site was a significantly increased incidence of neoplasms observed in dosed male or female mice.

Genetic Toxicology: PETN, NF, was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without exogenous metabolic activation (S9). When tested for cytogenetic effects in cultured CHO cells, PETN, NF, induced sister chromatid exchanges (SCEs) in the presence and absence of metabolic activation; no induction of chromosomal aberrations was observed in CHO cells with or without activation.

Audit: The data, documents, and pathology materials from the 2-year studies of PETN, NF, have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of PETN, NF, for male and female F344/N rats, based on a marginal increase in neoplasms of the Zymbal gland. Female rats might have tolerated a higher dose. There was *no evidence of carcinogenic activity* of PETN, NF, for male or female B6C3F$_1$ mice fed diets containing 25,000, or 50,000 ppm for 2 years. No nonneoplastic lesions were attributed to PETN, NF, administration.

Synonyms for PETN: 2,2-bis((nitrooxy)methyl)-1,3-propanediol dinitrate (ester); 2,2-bisdihydroxymethyl-1,3-propanediol tetranitrate; niperyt; nitropentaerythritol; pentaerythrityl tetranitrate; penthrit

Trade Names for PETN, NF: Angitet; Cardiacap; Dilcoran-80; Dipentrate; Hasethrol; Lentrat; Metranil; Mycardol; Neo-Corovas; Nitropenta; Nitropenton; Pen-

tafin; Pentanitrine; Pentitrate; Pentral 80; Pentrite; Pentritol; Pentryate; Peridex; Pergitral; Peritrate; Perityl; Prevangor; Quintrate; Subicard; Terpate; Vasodiatol

Report Date: August 1989

TR-366 Toxicology and Carcinogenesis Studies of Hydroquinone (CAS No. 123-31-9) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Hydroquinone is used an antioxidant in the rubber industry and as a developing agent in photography. It is also an intermediate in the manufacture of rubber and food antioxidants and monomer inhibitors. Hydroquinone and products containing hydroquinone are used as depigmenting agents to lighten skin. Toxicology and carcinogenesis studies were conducted by administering hydroquinone (greater than 99% pure) in corn oil or water by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Additionally, genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, Chinese hamster ovary (CHO) cells, and *Drosophila melanogaster*.

Preliminary 3-day dermal studies were conducted with rats and mice using sufficient hydroquinone in 95% ethanol to crystallize on the skin (4 or 40 mg per animal); conjugated metabolites of hydroquinone were detected in the urine. Fourteen-day dermal studies were conducted at doses up to 3,840 mg/kg for rats and 4,800 mg/kg for mice. No toxic effects were seen in the 3- or 14-day dermal studies. Therefore, in further evaluations of hydroquinone, the gavage route of administration was used.

Results of Fourteen-Day and Thirteen-Week Studies: Fourteen-day gavage studies were conducted by administering hydroquinone in corn oil to rats at doses ranging from 63 to 1,000 mg/kg body weight and to mice at doses ranging from 31 to 500 mg/kg. All rats receiving 1,000 mg/kg and 1/5 male and 4/5 female rats receiving 500 mg/kg died before the end of the 14 days. Compound-related clinical signs in rats included tremors lasting up to 30 minutes after each dosing at 500 and 1,000 mg/kg. In the 14-day gavage studies with mice, 4/5 male mice and 5/5 female mice receiving 500 mg/kg and 3/5 males receiving 250 mg/kg died before the end of the studies. Tremors followed by convulsions were seen at 250 and 500 mg/kg.

In the 13-week studies, doses for rats and mice ranged from 25 to 400 mg/kg. All rats receiving 400 mg/kg and 3/10 female rats receiving 200 mg/kg died before the end of the studies. The mean body weight at necropsy of male rats administered 100 or 200 mg/kg was about 8%-9% lower than that of vehicle controls. Mean body weights of vehicle control and dosed female rats at necropsy were similar. Tremors and convulsions were observed after dosing in most rats receiving 400 mg/kg and in several female rats receiving 200 mg/kg. Inflammation and/or epithelial hyperplasia (acanthosis) of the forestomach were seen in 4/10 male rats and 1/10 female rats receiving 200 mg/kg. Toxic nephropathy, characterized by tubular cell degeneration in the renal cortex, was seen in 7/10 male and 6/10 female rats receiving 200 mg/kg and in 1/10 females receiving 100 mg/kg.

In the 13-week studies in mice, 8/10 males and 8/10 females receiving 400 mg/kg and 2/10 male mice receiving 200 mg/kg died early. Mean body weights of dosed and vehicle control mice at necropsy were similar. Liver weight to body weight ratios for dosed male mice were significantly greater than for vehicle controls. Ulceration, inflammation, or epithelial hyperplasia of the forestomach was found in 3/10 male and 2/10 female mice receiving 400 mg/kg and 1/10 females receiving 200 mg/kg.

Based on these collective results, 2-year studies were conducted by administering 0, 25, or 50 mg/kg hydroquinone in deionized water by gavage to groups of 65 rats of each sex, 5 days per week. Groups of 65 mice of each sex were administered 0, 50, or 100 mg/kg on the same schedule. Ten rats and 10 mice from each group were killed after 15 months for an interim evaluation.

Observations at Fifteen Months: In the rats killed at 15 months, the relative kidney weight for high dose male rats was greater than that for vehicle controls. The hematocrit value, hemoglobin concentration, and erythrocyte count for high dose female rats were decreased. Compound-related increased severity of nephropathy was observed in male rats. In mice killed at 15 months, the relative liver weights for high dose male and female mice were significantly greater than those for vehicle controls. Lesions seen in the liver of male mice included increased syncytial cells and diffuse cytomegaly.

Body Weights, Organ Weights, and Survival in the Two-Year Studies: Mean body weights of high dose male rats were 5%-13% lower than those of vehicle controls after week 73, and those of low dose male rats were 5%-9% lower than those of vehicle controls after week 89. Mean body weights of dosed female rats were similar to those of vehicle controls throughout the study. The relative kidney and liver weights for high dose male rats were higher than those for vehicle controls. Mean body weights of high dose male mice were 5%-8% lower than those of vehicle controls after week 93, and those of high dose female mice were 5%-14% lower after week 20. Relative liver weights were increased for dosed male and high dose female mice. No significant differences in survival were observed between any groups of rats or mice of either sex after 2 years (male rats: vehicle control, 27/55; low dose, 18/55; high dose, 18/55; female rats: 40/55; 27/55; 32/55; male mice: 33/55; 37/54; 36/55; female mice: 37/55; 39/55; 36/55).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nearly all male rats and most female rats in all vehicle control and dosed groups had nephropathy. The severity of this disease was judged to be greater in high dose male rats. Hyperplasia of the renal pelvic transitional epithelium and renal cortical cysts, changes observed with advanced renal disease, were increased in

male rats. Renal tubular hyperplasia was seen in 2 high dose male rats, and renal tubular adenomas were seen in 4/55 low dose and 8/55 high dose male rats; none was seen in vehicle controls.

Mononuclear cell leukemia in female rats occurred with a positive trend, and the incidences in the dosed groups were greater than that in the vehicle controls (vehicle control, 9/55; low dose, 15/55; high dose, 22/55). The historical incidence of leukemia in water gavage vehicle control female F344/N rats is 25% ± 15% and in untreated controls is 19% ± 7%.

Compound-related lesions observed in the liver of high dose male mice included anisokaryosis (0/55; 2/54; 12/55), syncytial alteration (5/55; 3/54; 25/55), and basophilic foci (2/55; 5/54; 11/55). The incidences of hepatocellular adenomas were increased in dosed male mice (9/55; 21/54; 20/55), but these increases were offset by decreases in the incidences of hepatocellular carcinomas (13/55; 11/54; 7/55). The incidences of hepatocellular neoplasms, primarily adenomas, were increased in dosed female mice (3/55; 16/55; 13/55).

Follicular cell hyperplasia of the thyroid gland was increased in dosed mice (male: 5/55; 15/53; 19/54; female: 13/55; 47/55; 45/55). Follicular cell adenomas were seen in 2/55 vehicle control, 1/53 low dose, and 2/54 high dose male mice and in 3/55 vehicle control, 5/55 low dose, and 6/55 high dose female mice, a follicular cell carcinoma was seen in a seventh high dose female mouse. The highest observed incidence of follicular cell adenomas or carcinomas (combined) in historical water gavage vehicle control female $B6C3F_1$ mice is 3/48 (6%).

Genetic Toxicology: Hydroquinone was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation. It induced trifluorothymidine (Tft) resistance in mouse L5178Y/TK lymphoma cells in the presence or absence of metabolic activation. An equivocal response was obtained in tests for induction of sex-linked recessive lethal mutations in Drosophila administered hydroquinone by feeding. Hydroquinone induced sister chromatid exchanges (SCEs) in CHO cells both with or without exogenous metabolic activation and caused chromosomal aberrations in the presence of activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of hydroquinone for male F344/N rats, as shown by marked increases in tubular cell adenomas of the kidney. There was *some evidence of carcinogenic activity* of hydroquinone for female F344/N rats, as shown by increases in mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of hydroquinone for male $B6C3F_1$ mice administered 50 or 100 mg/kg in water by gavage. There was *some evidence of carcinogenic activity* of hydroquinone for female $B6C3F_1$ mice, as shown by increases in hepatocellular neoplasms, mainly adenomas.

Administration of hydroquinone was associated with thyroid follicular cell hyperplasia in both male and female mice and anisokaryosis, multinucleated hepatocytes, and basophilic foci of the liver in male mice.

Synonyms: 1,4-benzenediol; *p*-benzenediol; benzohydroquinone; benzoquinol; 1,4-dihydroxybenzene; *p*-dihydroxybenzene; *p*-dioxobenzene; *p*-dioxybenzene; hydroquinol; hydroquinole; α-hydroquinone; *p*-hydroquinone; *p*-hydroxyphenol; quinol; β-quinol

Report Date: October 1989

TR-367 Toxicology and Carcinogenesis Studies of Phenylbutazone (CAS No. 50-33-9) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Phenylbutazone is a nonsteroidal anti-inflammatory drug. Toxicology and carcinogenesis studies were conducted by administering phenylbutazone (greater than 99% pure) in corn oil by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 19 days, 13 weeks, or 2 years. Genetic toxicology studies were performed with *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Nineteen-Day Studies: The deaths of 3/5 male and 4/5 female rats that received 600 mg/kg and of 2/5 females that received 300 mg/kg were considered to be chemically related. The final mean body weight of rats that received 300 or 600 mg/kg was 14%-15% or 46% lower than that of vehicle controls. No compound-related deaths occurred in mice (doses up to 600 mg/kg). The final mean body weights of dosed and vehicle control mice were similar.

Thirteen-Week Studies: Most rats that received 300 mg/kg and 1/10 male and 2/10 female rats that received 200 mg/kg died early. The final mean body weight of male rats at 300 mg/kg was 31% lower than that of the vehicle controls. The liver weight to body weight ratios were increased in the 200 and 300 mg/kg group of rats. Compound-related lesions occurred mainly in the kidney and included papillary necrosis, papillary edema, and multifocal mineralization.

Five of 10 male mice and 4/10 female mice that received 600 mg/kg died early. No other compound-related deaths occurred in mice. Final mean body weights of dosed and vehicle control mice were comparable. The liver weight to body weight ratios were increased for mice at 300 and 600 mg/kg. No compound-related histopathologic effects were observed in mice.

Body Weight and Survival in the Two-Year Studies: Two-year studies were conducted by administering 0, 50, or 100 mg/kg phenylbutazone in corn oil by gavage to groups of 50 rats of each sex, 5 days per week for 103 weeks. The doses given groups of 50 mice of each sex on the same schedule were 0, 150, or 300 mg/kg. Mean body weights of high dose rats were generally 6%-11% lower than those of vehicle controls. Mean body weights of mice were similar among all groups except for high dose female mice, which weighed 4%-11% less than vehicle controls. The survival of all groups was similar except for that of the low dose group of male rats, which was significantly lower than that of the vehicle controls at the

end of the studies; the survival of the top dose group of female rats and the vehicle control group of female mice was low but not statistically reduced (final survival—male rats: vehicle control, 33/50; low dose, 20/50; high dose, 27/50; female rats: 31/50; 35/50; 22/50; male mice: 36/50; 40/50; female mice: 22/50; 29/50; 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Mild pyelonephritis, renal papillary necrosis, and mineralization of the renal papillae in dosed male and female rats and hyperplasia of the renal pelvis epithelium, dilatation of the renal pelvis, and renal cysts in dosed female rats were observed at increased incidences compared with those in vehicle controls. A renal tubular cell carcinoma was observed in one low dose male rat, and renal tubular adenomas were observed in three high dose male rats. A carcinoma of uncertain histogenesis was observed in one low dose female rat. Carcinomas of the renal transitional epithelium were seen in two high dose female rats. When the kidneys were step-sectioned, additional tubular cell adenomas were diagnosed in four low dose and one high dose male rats and in three low dose and one high dose female rats; none was observed in vehicle controls.

Papillomas of the transitional epithelium of the urinary bladder were seen in 2/43 low dose male and 1/49 low dose female F344/N rats. The historical incidence of urinary bladder transitional cell neoplasms in male corn oil vehicle control F344/N rats is 5/2,034 (0.2%; highest observed incidence, 2/50) and 4/2,026 (0.2%; highest observed incidence, 1/45) in females.

Adrenal medullary hyperplasia was observed at an increased incidence in high dose female rats (vehicle control, 3/50; low dose, 6/50; high dose, 19/50).

Ulcers of the forestomach were observed at increased incidences in high dose rats (male: 0/50; 5/50; 6/50; female: 2/49; 1/49; 12/49). In high dose female rats, acanthosis (4/49; 0/49; 12/49), hyperkeratosis (3/49; 0/49; 12/49), and basal cell hyperplasia (4/49; 1/49; 12/49) of the forestomach were observed at increased incidences. No neoplasms were associated with these stomach lesions.

Peliosis hepatis, centrilobular cytomegaly and karyomegaly, fatty change, hepatocellular degeneration, and coagulative necrosis of the liver were observed in dosed male mice; clear cell foci were observed in five high dose male mice. The incidences of hepatocellular adenomas and adenomas or carcinomas (combined) in male mice were increased in the high dose group (adenomas or carcinomas, combined: 16/50; 14/50; 31/50).

Genetic Toxicology: Phenylbutazone was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without exogenous metabolic activation. Phenylbutazone produced a positive response in the mouse lymphoma assay in both the presence and absence of activation. Phenylbutazone induced chromosomal aberrations in CHO cells in the presence, but not the absence, of exogenous metabolic activation; no induction of sister chromatid exchanges was observed in CHO cells in the presence or absence of activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity* of phenylbutazone for male F344/N rats, as shown by the occurrence of small numbers of renal tubular cell adenomas or carcinomas. There was *some evidence of carcinogenic activity* for female F344/N rats, as shown primarily by the occurrence of two rare transitional cell carcinomas in the top dose group; none has ever been seen in vehicle control or untreated control female rats. Tubular cell adenomas may have been associated with the administration of phenylbutazone to female rats. There was *some evidence of carcinogenic activity* for male $B6C3F_1$ mice, as shown by the increased incidence of hepatocellular adenomas or carcinomas (combined). There was *no evidence of carcinogenicity* for female $B6C3F_1$ mice administered phenylbutazone in corn oil by gavage at doses of 150 or 300 mg/kg.

Phenylbutazone was also nephrotoxic to rats, as shown by the dose-related increase in the severity of age-related nephropathy, necrosis of the renal papilla, and mineralization of the collecting ducts in the papilla.

Synonyms: 4-butyl-1,2-diphenyl-3,5-pyrazolidinedione; 3,5-dioxo-1,2-diphenyl-4-*n*-butylpyrazolidine

Trade Names: There have been over 100 registered trade names including: Anerval; Azobutil; Bizolin 200; Butacote; Butadion; Butagesic; Butazolidin; Chembutazone; Equi Bute; Flexazone; Fenibutol; G 13,871; Pyrazolidin; Reumazol; Robizon-V; Uzone

Report Date: March 1990

TR-368 Toxicology and Carcinogenesis Studies of Nalidixic Acid (CAS No. 389-08-2) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Nalidixic acid is an antimicrobial agent to treat bacterial infections of the urinary tract. Toxicology and carcinogenesis studies were conducted by feeding diets containing nalidixic acid (approximately 99% pure) to groups of F344/N rats and $B6C3F_1$ mice of each sex for 13 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells.

Thirteen-Week Studies: Nalidixic acid was administered at dietary concentrations ranging from 1,000 to 16,000 ppm. One female rat that received 16,000 ppm nalidixic acid died before the end of the studies; no other compound-related deaths occurred in rats and mice. The final mean body weights of rats that received 8,000 or 16,000 ppm were 23% or 49% lower than those of controls for males and 11% or 31% lower for females. Feed consumption by rats receiving 16,000 ppm was approximately two-thirds that by controls. Liver weight to body weight ratios for male rats that received 2,000 ppm or more and female rats that received 8,000 ppm or more were significantly greater than those for controls. Degeneration of the germinal epithelium in the semi-

niferous tubules of the testis was observed in 10/10 male rats that received 16,000 ppm; no other compound-related histopathologic effects were observed in rats. The final mean body weights of mice that received 8,000 or 16,000 ppm were 10%-20% lower than those of controls. Feed consumption by dosed mice was similar to that by controls. Liver weight to body weight ratios were significantly greater for male mice receiving 2,000, 8,000, or 16,000 ppm and for female mice receiving 4,000, 8,000, or 16,000 ppm than for the controls. No compound-related histopathologic effects were observed in mice.

Based on these results, 2-year studies of nalidixic acid were conducted by feeding diets containing 0, 2,000, or 4,000 ppm nalidixic acid to groups of 50 male and 50 female F344/N rats and 50 male and 50 female $B6C3F_1$ mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose rats were 7%-23% lower than those of controls, and those of low dose male rats were 6%-11% lower than those of controls. The average daily feed consumption by dosed rats ranged from 89% to 96% that by controls. The average amount of nalidixic acid consumed per day was approximately 80 or 175 mg/kg for low dose or high dose rats. Mean body weights of high dose male mice were 1%-8% lower than those of controls throughout the study. Mean body weights of dosed female mice were 5%-17% lower than those of controls. Average daily feed consumption by dosed mice was within 3% of that by controls. The estimated average amount of nalidixic acid consumed per day was approximately 220 or 475 mg/kg for low dose or high dose mice. No significant differences in survival were seen between any groups of rats or mice of either sex after 2 years (male rats: control, 27/50; low dose, 28/50; high dose, 27/50; female rats: 22/50; 31/50; 29/50; male mice: 33/50; 34/50; 31/50; female mice: 40/50; 43/50; 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The incidences of preputial gland neoplasms in dosed male rats and of clitoral gland neoplasms in dosed female rats were significantly greater than those in controls (male—preputial gland adenomas, papillomas, or carcinomas, combined: control, 3/49; low dose, 19/49; high dose, 20/47; female—clitoral gland adenomas, papillomas, or carcinomas, combined: 5/46; 15/46; 16/47).

A squamous cell carcinoma of the tongue was seen in two high dose male rats. The historical incidence of oral cavity neoplasms in untreated control male F344/N rats is 7/1,596 (0.4%).

There were decreased incidences of leukemia (20/50; 9/50; 7/50) and mammary gland neoplasms (10/50; 7/50; 2/50) in dosed female rats and of pituitary gland neoplasms (11/49; 2/50; 2/50) in dosed male rats.

Retinal degeneration and cataracts of the eye were observed at increased incidences in dosed rats (degeneration—male: 4/48; 41/48; 47/49; female: 2/47; 40/48; 46/50; cataracts—male: 11/48; 23/48; 38/49; female: 0/47; 18/48; 14/50). The cause of these cataracts and retinal degeneration is uncertain because cages were not rotated and low and high dose groups of rats may have been exposed to greater light intensity than were the controls.

Subcutanous tissue fibrosarcomas and fibromas or fibrosarcomas (combined) were increased in dosed male mice (fibromas or fibrosarcomas, combined: 5/50; 9/50; 14/50). There were no increased incidences of neoplasms in dosed female mice.

Genetic Toxicology: Nalidixic acid was not mutagenic in any of several in vitro short-term tests. No gene reversion was observed in *S. typhimurium* strains TA97, TA98, TA100, or TA1535 after exposure to nalidixic acid in either the presence or absence of exogenous metabolic activation. Results of tests for induction of trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells were negative with or without metabolic activation. In CHO cells, nalidixic acid did not induce sister chromatid exchanges or chromosomal aberrations in either the presence or absence of activation.

Conclusions: Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity* of nalidixic acid for F344/N rats, as indicated by increased incidences of preputial gland neoplasms in males and clitoral gland neoplasms in females. There was *equivocal evidence of carcinogenic activity* for male $B6C3F_1$ mice fed diets containing nalidixic acid, as indicated by marginally increased incidences of subcutaneous tissue neoplasms. There was *no evidence of carcinogenic activity* for female $B6C3F_1$ mice fed diets containing 2,000 or 4,000 ppm nalidixic acid for 2 years.

Synonym: 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid

Trade Names: NegGram®; Dixiben®; Nalidixan®; Nalurin®; Nogram®; UroNeg®; Uralgin®; Urisal®

Report Date: October 1989

TR-369 Toxicology and Carcinogenesis Studies of α-Methylbenzyl Alcohol (CAS No. 98-85-1) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of α-methylbenzyl alcohol (greater than 99% pure), a cosmetic ingredient and food flavoring agent, were conducted by administering the chemical in corn oil by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells. α-Methylbenzyl alcohol was nominated for study by the National Cancer Institute because of the potential for widespread human exposure.

Sixteen-Day and Thirteen-Week Studies: The doses used in the 16-day studies for rats and mice ranged between 125 and 2,000 mg/kg. Six of 10 rats and all mice dosed at 2,000 mg/kg died. In addition, because 7/9 mice dosed at 1,000 mg/kg died, the doses selected for the 13-week studies for mice (47-750 mg/kg) were half those used for rats (93-1,500 mg/kg).

In the 13-week studies, deaths of 1/10 male and 3/10 female rats dosed at 1,500 mg/kg were compound related; none of the mice died. Body weight gain was reduced in rats at 1,500 mg/kg; there were no significant histopathologic lesions in either rats or mice. The only compound-related effects were ataxia, labored breathing, and lethargy for up to 30 minutes after dosing in rats and mice given the two highest doses and increases in liver weight to body weight ratios for male rats given the three highest doses and for female rats at all doses.

Based on the pattern of mortality and the effects on body weight gain in the short-term studies, doses of 375 and 750 mg/kg α-methylbenzyl alcohol were administered in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 rats and 50 mice of each sex.

Two-Year Studies: Significant reduction in body weight gain commenced at weeks 20-30 in high dose male and female rats, and body weights were 20%-30% below those of vehicle controls at study termination. In the low dose groups, body weight reduction occurred only in male rats during the last 10 weeks of the study. After 80 weeks, 60% of the high dose rats and 80%-100% of the low dose and vehicle control rats were alive; thereafter, the number of deaths in the chemically exposed groups increased sharply so that, at the end of 2 years, final survival for vehicle control, low dose, and high dose rats was 35/50; 8/50; and 1/50 for males and 34/50, 25/50, and 11/50 for females. There were a large number of gavage accidents in these studies (1, 9, and 8 for male rats and 1, 4, and 14 for female rats), but these accidents did not contribute to the increase in mortality after week 80, as all but 4 of these occurred earlier.

Mortality in the last quarter of the study was thought to be due to the effects of cumulative toxicity of α-methylbenzyl alcohol on a renal excretory system already compromised by aging. Renal nephropathy that commonly occurs during aging was found in all groups of rats, but the severity was greater in male rats dosed with α-methylbenzyl alcohol. In addition, a collection of nonneoplastic lesions (parathyroid hyperplasia, calcification of the heart and glandular stomach, and fibrous osteodystrophy of bone) was found in the dosed male rats; these lesions were probably secondary to mineral imbalance arising from renal dysfunction.

Since survival was poor in low and high dose male and high dose female rats, the sensitivity of the study for detecting a carcinogenic effect in these groups was reduced. Despite this limitation, there were dose-related increases in the incidences of renal tubular cell adenomas or adenocarcinomas (combined) in male rats (vehicle control, 0/50; low dose, 2/50; high dose, 5/50). In addition, transitional cell papillomas of the urinary bladder were observed in one high dose male and two high dose female rats.

In mice, a reduction in body weight gain was apparent in the high dose groups of males and females. Final survival rates in mice were similar among groups (male: 39/49; 40/50; 28/50; female: 41/50; 41/50; 38/50). No neoplastic or nonneoplastic lesions were attributed to α-methylbenzyl alcohol administration in mice of either sex.

Genetic Toxicology: α-Methylbenzyl alcohol was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in the presence or absence of exogenous metabolic activation. α-Methylbenzyl alcohol produced a positive response without activation in the mouse L5178Y/TK$^{+/-}$ lymphoma assay for induction of trifluorothymidine resistance; it was not tested with activation. In cytogenetic tests with CHO cells, α-methylbenzyl alcohol induced chromosomal aberrations in the presence, but not the absence, of metabolic activation; no induction of sister chromatid exchanges was observed in CHO cells after exposure to α-methylbenzyl alcohol.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of α-methylbenzyl alcohol for male F344/N rats, as shown by increased incidences of renal tubular cell adenomas and adenomas or adenocarcinomas (combined). There was *no evidence of carcinogenic activity* for female F344/N rats administered 375 or 750 mg/kg. Renal toxicity characterized by severe nephropathy and related secondary lesions was observed in the dosed rats, and excessive mortality occurred during the last quarter of the studies. Poor survival reduced the sensitivity of the studies for detecting the presence of a carcinogenic response both in chemically exposed groups of male rats and in the high dose group of female rats. There was *no evidence of carcinogenic activity* of α-methylbenzyl alcohol for male or female B6C3F$_1$ mice administered 375 or 750 mg/kg for 2 years.

Synonyms: styrallyl alcohol; styralyl alcohol; α-methylbenzenemethanol; phenylmethylcarbinol; 1-phenethyl alcohol

Report Date: January 1990

TR-370 Toxicology and Carcinogenesis Studies of Benzofuran (CAS No. 271-89-6) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Benzofuran is used as an intermediate in the polymerization of coumarone-indene resins found in various corrosion-resistant coatings such as paints and varnishes, in water-resistant coatings for paper products and fabrics, and in adhesives approved for use in food containers. Toxicology and carcinogenesis studies were conducted by administering benzofuran (approximately 99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology tests were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: Benzofuran doses for groups of five rats ranged from 63 to 1,000 mg/kg and from 16 to 250 mg/kg for mice. All male and female rats that received 1,000 mg/kg and one female rat that received 500 mg/kg died before the end of the studies. The final mean

body weights of male rats that received 250 or 500 mg/kg were 13% or 21% lower than that of controls; the final mean body weight of female rats that received 500 mg/kg was 10% lower than that of controls. Final mean body weights of chemically exposed and control mice were similar. No compound-related histologic lesions were found in rats or mice.

Thirteen-Week Studies: Doses for groups of 10 rats and 10 mice ranged from 31 to 500 mg/kg. One female rat that received 500 mg/kg and one that received 250 mg/kg died before the end of the study. Final mean body weights of male rats that received 125, 250, or 500 mg/kg were 11%, 17%, or 27% lower than that of vehicle controls; the final mean body weight of female rats that received 500 mg/kg was 11% lower than that of vehicle controls. Histologic lesions observed in chemically exposed rats included minimal hepatocellular necrosis, increased severity of nephropathy, and cytoplasmic vacuolization of the adrenal cortex.

Seven male and three female mice that received 500 mg/kg and one male mouse that received 250 mg/kg died before the end of the 13-week studies. The final mean body weight of mice that received 500 mg/kg was 13% lower than that of vehicle controls. Nephrosis was observed in male mice that received 250 mg/kg.

Based on reduced mean body weights, increased severity of nephropathy, and hepatocellular necrosis, benzofuran doses selected for the 2-year studies in rats were 30 or 60 mg/kg for males and 60 or 120 mg/kg for female. Based on increased mortality and nephrosis in male mice, doses selected for the 2-year studies in mice were 60 or 120 mg/kg for males and 120 or 240 mg/kg for females.

Body Weights and Survival in the Two-Year Studies: Mean body weights of high dose rats and dosed male mice were 4%-11% lower than those of vehicle controls. Mean body weights of chemically exposed female mice were 8%-35% lower than those of vehicle controls. The survival of chemically exposed male rats was reduced after week 92 (survival at week 89: vehicle control, 47/50; low dose, 39/50; high dose, 38/50; final survival: vehicle control, 33/50; low dose, 12/50; high dose, 18/50). Survival of chemically exposed female rats and male mice was similar to that of vehicle controls after 2 years (female rats: 27/50; 23/50; 25/50; male mice: 33/50; 20/50; 28/50). Deaths of 10 low dose male mice at weeks 20-21 were caused by a dosing error; these animals were not included in survival and tumor analyses. Survival of chemically exposed female mice was reduced after week 89 (final survival: 37/50; 19/50; 21/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nephropathy occurred with increased severity in chemically exposed male rats. The incidences of parathyroid hyperplasia, fibrous osteodystrophy, mineralization of the pulmonary artery, renal cortical cysts, and hyperplasia of the pelvic epithelium were increased in chemically exposed male rats. The incidence of nephropathy was increased in chemically exposed female rats (vehicle control, 29/50; low dose, 48/50; high dose, 39/50). Renal atypical tubular cell hyperplasia and renal tubular cell adenocarcinomas occurred in chemically exposed female rats (atypical tubular cell hyperplasia: 0/50; 1/50; 3/50; tubular cell adenocarcinomas: 0/50; 1/50; 4/50). No renal tubular cell adenocarcinomas have been observed in 2,094 female corn oil vehicle control F344/N rats in National Toxicology Program studies.

Chronic inflammation, ulcers, and epithelial hyperplasia of the forestomach were observed at increased incidences in chemically exposed male rats (chronic inflammation: 1/50; 11/50; 6/49; ulcers: 1/50; 5/50; 8/49; epithelial hyperplasia: 9/50; 15/50; 18/49).

Metaplastic hepatocytes arising within pancreatic islets occurred at an increased incidence in high dose female rats (0/50; 1/50; 11/49).

The incidences of neurilemomas were markedly increased above the historical control incidences (0.1%-0.4%) in all groups of rats (male: 18/50; 13/50; 14/50; female: 7/50; 9/50; 3/50).

Syncytial alteration of the liver occurred at increased incidences in male mice exposed to benzofuran. The incidences of hepatocellular adenomas, hepatoblastomas (high dose male mice) and hepatocellular adenomas, hepatocellular carcinomas, or hepatoblastomas (combined) were increased in chemically exposed mice (male—adenomas: 4/49; 24/39; 34/48; hepatoblastomas: 0/49; 3/39; 18/48; carcinomas, adenomas, or hepatoblastomas, combined: 12/49; 31/39; 40/48; female—adenomas: 1/50; 22/48; 21/47; hepatoblastomas: 0/50; 1/48; 2/47; carcinomas, adenomas, or hepatoblastomas, combined: 4/50; 25/48; 22/47).

Squamous cell papillomas or carcinomas (combined) of the forestomach were increased in chemically exposed mice (male: 2/49; 11/39; 13/48; female: 2/50; 9/50; 5/50).

The incidences of epithelial hyperplasia of the bronchioles were increased in chemically exposed mice. The incidences of alveolar/bronchiolar adenomas or carcinomas (combined) in high dose males and chemically exposed females were increased (adenomas or carcinomas, combined—male: 10/49; 9/39; 19/48; female: 2/50; 9/48; 14/47).

Genetic Toxicology: Benzofuran was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of exogenous metabolic activation. Benzofuran induced trifluorothymidine resistance in mouse L5178Y lymphoma cells treated in the absence of metabolic activation; this assay was not conducted with activation. Benzofuran induced sister chromatid exchanges but not chromosomal aberrations in CHO cells in the presence and absence of activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of benzofuran for male F344/N rats receiving doses of 30 or 60 mg/kg per day. There was *some evidence of carcinogenic activity* of benzofuran for female F344/N rats, based on increased incidences of tubular cell adenocarcinomas of the kidney. There was *clear evidence of carcinogenic activity* for male and female $B6C3F_1$ mice, based on increased incidences of neoplasms of the liver, lung, and forestomach.

Exposure to benzofuran increased the severity of nephropathy in male rats, increased the incidences of

nephropathy in female rats, and induced hepatocellular metaplasia in the pancreas in female rats. Nonneoplastic lesions observed in mice exposed to benzofuran included syncytial alteration of the liver, bronchiolar epithelial hyperplasia, and epithelial hyperplasia of the forestomach.

Synonyms: coumarone; cumarone

Report Date: October 1989

TR-371 Toxicology and Carcinogenesis Studies of Toluene (CAS No. 108-88-3) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

Toluene is used to back-blend gasoline, as a chemical intermediate, and as a solvent; 920 million gallons were produced in the United States in 1988. Toxicology studies were conducted by administering toluene (greater than 99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 13 weeks or by whole-body inhalation exposure for 14 or 15 weeks. Toxicology and carcinogenesis studies were conducted by whole-body inhalation exposure of F344/N rats and B6C3F$_1$ mice of each sex for 15 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary cells.

Thirteen-Week Gavage Studies: All rats that received the top dose of 5,000 mg/kg died during the first week, and 8/10 male rats that received 2,500 mg/kg died early. The final mean body weight of male rats that received 2,500 mg/kg was 19% lower than that of vehicle controls. Relative liver, kidney, and heart (female only) weights for rats that received the higher doses were greater than those for vehicle controls. Necrosis of the brain and hemorrhage of the urinary bladder were seen at increased incidences in dosed rats.

All mice that received the top dose of 5,000 mg/kg died during the first week, and 40% of those that received 2,500 mg/kg died before the end of the 13-week gavage studies. The final mean body weight of males at 2,500 mg/kg was 16% lower than that of vehicle controls. At the higher doses, relative liver weights were increased for mice.

Fifteen-Week and Fourteen-Week Inhalation Studies: Eight of 10 male rats exposed at the top exposure concentration of 3,000 ppm died during week 2. Final mean body weights of rats exposed at concentrations of 2,500 or 3,000 ppm were 14%-25% lower than that of controls. As in the gavage studies, the relative liver, kidney, and heart weights for rats exposed at the top two concentrations were increased compared with those for controls. No compound-related effects were seen on sperm; no adverse effects on the estrous cycle were observed.

Five of 10 male mice and all female mice exposed at 3,000 ppm and 70% of female mice at 2,500 ppm died during the first 2 weeks. Final mean body weights of all exposed groups were 7%-13% lower than those of controls. Relative liver weights for mice exposed at 625 ppm or higher, relative lung weights for mice exposed at 1,250 ppm or higher, and relative kidney weights for female mice exposed at 1,250 ppm or higher were greater than those for controls. Centrilobular hypertrophy of the liver was observed in all male mice exposed at 2,500 ppm and 70% of male mice exposed at 3,000 ppm. No effects on sperm or the estrous cycle were observed.

Fifteen-Month and Two-Year Inhalation Studies: Long-term studies were conducted by exposing groups of 60 rats of each sex to 0, 600, or 1,200 ppm toluene by inhalation, 6.5 hours per day, 5 days per week. Groups of 60 mice of each sex were exposed at 0, 120, 600, or 1,200 ppm on the same schedule. Ten animals per group (except male mice) were removed for toxicologic evaluation after being exposed for 15 months. All other animals were exposed to toluene for 103 weeks.

In the 15-month inhalation studies, the incidences and severity of nonneoplastic lesions of the nasal cavity (degeneration of olfactory and respiratory epithelium and goblet cell hyperplasia) were increased in exposed rats. Minimal hyperplasia of the bronchial epithelium was seen in 4/10 female mice at 1,200 ppm. The severity of nephropathy was slightly increased in exposed female rats. No chemical-induced neoplasms were observed.

Body Weight and Survival in the Two-Year Studies: Mean body weights of rats and mice were generally similar (yearly averages within 5%) among groups throughout the 2-year studies. No significant differences in survival were observed among rats or mice of either sex, although survival in all groups of male mice was lower than usual (male rats: control, 30/50; 600 ppm, 28/50; 1,200 ppm, 22/50; female rats: 33/50; 35/50; 30/50; male mice: control, 17/60; 120 ppm, 22/60; 600 ppm, 16/60; 1,200 ppm, 19/60; female mice: 30/50; 33/50; 24/50; 32/50). Scrotal, preputial, and penile lesions observed in male mice were associated with many of the early deaths and with animals killed in a moribund condition.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nephropathy was seen in almost all rats, and the severity was somewhat increased in exposed rats. A rare renal tubular cell carcinoma in a female rat and an equally uncommon sarcoma of the kidney in another female rat were seen in the 1,200-ppm exposure group. Erosion of the olfactory epithelium and degeneration of the respiratory epithelium were increased in exposed rats. Inflammation of the nasal mucosa and metaplasia of the olfactory epithelium were increased in exposed female rats. A rare squamous cell carcinoma of the nasal mucosa was seen in one female rat at 1,200 ppm. A squamous cell papilloma of the forestomach was observed in one female rat at 1,200 ppm, and a squamous cell carcinoma was observed in a second female rat at 1,200 ppm. No chemically related neoplasms were found in male rats, and the one nasal, two kidney, and two forestomach neoplasms observed in female rats were considered not to be associated with inhalation exposed to toluene.

For mice, no biologically important increases were observed for any nonneoplastic or neoplastic lesions.

Genetic Toxicology: Toluene did not induce gene mutations in *S. typhimurium* strain TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation. In the mouse lymphoma assay, toluene gave an equivocal response with and without exogenous metabolic activation. Toluene did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of exogenous metabolic activation.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity* for male or female F344/N rats exposed to toluene at concentrations of 600 or 1,200 ppm. There was *no evidence of carcinogenic activity* for male or female $B6C3F_1$ mice exposed by inhalation to toluene at concentrations of 120, 600, or 1,200 ppm for 2 years.

Synonyms: monomethylbenzene; methylbenzene; toluol; phenylmethane; tolueen (Dutch); toluen (Czech), tolueno (Spanish); toluolo (Italian)

Trade Name: Methacide

Report Date: February 1990

TR-372 Toxicology and Carcinogenesis Studies of 3,3'-Dimethoxybenzidine Dihydrochloride (CAS No. 20325-40-0) in F344/N Rats (Drinking Water Studies)

3,3'-Dimethoxybenzidine dihydrochloride is an off-white powder with a melting point of 274° C. 3,3'-Dimethoxybenzidine is used principally as an intermediate in the production of commercial bisazobiphenyl dyes for coloring textiles, paper, plastic, rubber, and leather. In the synthesis of the bisazobiphenyl dyes, the amine groups of 3,3'-dimethoxybenzidine are chemically linked with other aromatic amines. A small quantity of 3,3'-dimethoxybenzidine is also used as an intermediate in the production of *o*-dianisidine diisocyanate, which is used in isocyanate-based adhesive systems and as a component of polyurethane elastomers.

3,3'-Dimethoxybenzidine dihydrochloride was evaluated in toxicity and carcinogenicity studies as part of the National Toxicology Program's Benzidine Dye Initiative. This Initiative was designed to evaluate the representative benzidine congeners and benzidine congener-derived and benzidine-derived dyes. 3,3'-Dimethoxybenzidine dihydrochloride was nominated for study because of the potential for human exposure during production of bisazobiphenyl dyes and because benzidine, a structurally related chemical, is a known human carcinogen.

Toxicology and carcinogenesis studies were conducted by administering 3,3'-dimethoxybenzidine dihydrochloride (greater than 97.5% pure) in drinking water to groups of F344/N rats of each sex for 14 days, 13 weeks, 9 months, or 21-months. The 21-month studies were intended to last 24 months but were terminated early because of rapidly declining survival due to neoplasia. Studies were performed only in rats because similar studies are being performed in mice at the National Center for Toxicology Research. Genetic toxicology studies were conducted with *Salmonella typhimurium*, Chinese hamster over (CHO) cells, and *Drosophila melanogaster*.

Fourteen-Day Studies: All rats receiving drinking water concentrations up to 4,500 ppm lived to the end of the studies. Rats that received water containing 4,500 ppm 3,3'-dimethoxybenzidine dihydrochloride lost weight. Water consumption decreased with increasing concentration of chemical and at 4,500 ppm was less than one-fourth that by the controls. Lymphoid depletion of the thymus in males and hypocellularity of the bone marrow in males and females were seen at the 4,500-ppm concentration, but not at the next lower concentration or in controls.

Thirteen-Week Studies: All rats receiving concentrations up to 2,500 ppm lived to the end of the studies. Final mean body weights of rats given drinking water containing 1,250 or 2,500 ppm 3,3'-dimethoxybenzidine dihydrochloride were 5%-20% lower than those of controls. Water consumption at these concentrations was 40%-60% that consumed by controls. Compound-related effects in rats given water containing 2,500 ppm 3,3'-dimethoxybenzidine dihydrochloride included a mind exacerbation of naturally occurring nephropathy and the presence of a yellow-brown pigment (lipofuscin) in the cytoplasm of thyroid follicular cells. Serum triiodothyronine (T_3) and thyroxin (T_4) concentrations in females receiving 330 ppm or more and T_4 concentrations in males receiving 170 ppm or more were significantly lower than in controls. Thyrotropin (TSH) concentrations were comparable in controls and exposed rats.

Based on the chemical-related nephropathy and reductions in water consumption and body weight gain observed in the 13-week studies, doses for the long-term studies in male and female rats were 0 or 330 ppm 3,3'-dimethoxybenzidine dihydrochloride in drinking water administered for 9 months and 0, 80, 170, or 330 ppm administered for 21 months.

Nine-Month Studies: Ten rats of each sex in control and 330-ppm groups were evaluated after 9 months. Significant decreases in T_3 and T_4 concentrations were seen in exposed male and female rats. Other lesions seen in exposed rats included foci of alteration in the liver, a carcinoma of the preputial gland in one male, a carcinoma of the clitoral gland in one female, and carcinoma of the Zymbal gland in two males.

Body Weights and Survival in the Twenty-One-Month Studies: The average amount of 3,3'-dimethoxybenzidine dihydrochloride consumed per day was approximately 6, 12, or 21 mg/kg for low, mid, or high dose male rats and 7, 14, or 23 mg/kg for low, mid, or high dose female rats. Mean body weights of male and female rats began to decrease relative to those of controls after about 1 year of exposure at 170 or 330 ppm and were 6%-22% lower for males and 7%-17% lower for females. Survival of rats exposed to 3,3'-dimethoxybenzidine dihydrochloride was reduced because animals were dying with neoplasms or being killed in a moribund condition (survival at 21

months—male: control, 44/60, 73%; low dose, 8/45, 18%; mid dose, 0/75; high dose, 0/60; female: 45/60, 75%; 15/45, 33%; 6/75, 8%; 0/60). Because of these early compound-related deaths, the studies were terminated at 21 months.

Nonneoplastic and Neoplastic Effects in the Twenty-One-Month Studies: Increased incidences of several nonneoplastic lesions were observed in exposed rats, including hematopoietic cell proliferation in the spleen and cystic and centrilobular degeneration and necrosis of the liver. Neoplasms attributed to 3,3'-dimethoxybenzidine dihydrochloride exposure were observed in rats at many tissue sites, including the skin, Zymbal gland, preputial and clitoral glands, oral cavity, small and large intestines, liver, brain, mesothelium, mammary gland, and uterus/cervix. The incidences of these neoplasms in male and female rats are given in the abstract summary table (see page 5 of the Technical Report).

Genetic Toxicology: 3,3'-Dimethoxybenzidine was mutagenic in *S. typhimurium* strain TA100 with exogenous metabolic activation and in strain TA98 without activation; a weakly positive response was observed in strain TA1535 with metabolic activation. 3,3'-Dimethoxybenzidine induced sister chromatid exchanges and chromosomal aberrations in CHO cells with and without exogenous metabolic activation. 3,3'-Dimethoxybenzidine did not induce sex-linked recessive lethal mutations in adult male *D. melanogaster* exposed via feeding or injection.Conclusions: Under the conditions of these 21-month drinking water studies, there was *clear evidence of carcinogenic activity* of 3,3'-dimethoxybenzidine dihydrochloride for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal gland, preputial gland, oral cavity, intestine, liver, and mesothelium. Increased incidences of astrocytomas of the brain may have been related to chemical administration. There was *clear evidence of carcinogenic activity* of 3,3'-dimethoxybenzidine dihydrochloride for female F344/N rats, as indicated by benign and malignant neoplasms of the Zymbal gland, clitoral gland, and mammary gland. Increases in neoplasms of the skin, oral cavity, large intestine, liver, and uterus/cervix were also considered to be related to chemical administration of 3,3'-dimethoxybenzidine dihydrochloride.

Synonyms: *o*-dianisidine dihydrochloride; 3,3'-dimethoxy-1,1-biphenyl)-4,4'-diamine dihydrochloride; 3,3'-dimethoxy-4,4'-diaminobiphenyl dihydrochloride

Report Date: January 1990

TR-373 Toxicology and Carcinogenesis Studies of Succinic Anhydride (CAS No. 108-30-5) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Succinic anhydride, a food additive, is also used in the manufacture of polymeric materials, pharmaceuticals, and agricultural and industrial chemicals. Toxicology and carcinogenesis studies were conducted by administering suspensions of succinic anhydride (97% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 or 20 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Twenty-Day or Sixteen-Day and Thirteen-Week Studies: In the 20-day studies in rats, doses of succinic anhydride given on 14 exposure days ranged from 47 to 750 mg/kg. Compound-related deaths occurred in the groups of males that received 375 mg/kg or higher doses and in females that received 187 mg/kg or higher doses. Necrosis and inflammation of the upper respiratory tract were seen in 3/10 male and 3/10 female rats given 750 mg/kg and 2/10 female rats given 375 mg/kg.

In the 16-day studies in mice, doses of succinic anhydride given on 12 exposure days ranged from 219 to 3,500 mg/kg. All animals that received 875 mg/kg or higher doses of succinic anhydride died before the end of the studies. No compound-related lesions were seen in male or female mice examined from the 438 mg/kg dose group.

In the 13-week studies in rats, doses of succinic anhydride ranged from 25 to 400 mg/kg for males and from 12.5 to 200 mg/kg for females. Deaths of 8/10 male rats that received 400 mg/kg and 4/10 males and 5/10 females that received 200 mg/kg were compound related. At necropsy, the mean body weights of male rats that received 200 or 400 mg/kg were 9% or 15% lower than that of vehicle controls, whereas the mean body weights of dosed and vehicle control female rats were similar. No compound-related gross or microscopic lesions were observed.

In the 13-week studies in mice, doses of succinic anhydride ranged from 37 to 600 mg/kg. All 10 males and 8/10 females that received 600 mg/kg and 2/10 males and 2/10 females that received 300 mg/kg died before the end of the studies. The final mean body weights of mice that received 150 or 300 mg/kg were 13% or 9% lower than that of vehicle controls for males and 8% or 7% lower for females. Mild inflammation of the stomach was observed in 7/10 male mice that received 150 mg/kg and 5/10 males that received 300 mg/kg compared with 2/10 vehicle controls.

Based primarily on the effects of administration of succinic anhydride on survival and mean body weights of rats and mice, doses for the 2-year studies were 0, 50, or 100 mg/kg to groups of 60 rats of each sex; 0, 38, or 75 mg/kg to groups of 50 male mice; and 0, 75, or 150 mg/kg to groups of 50 female mice. Succinic anhydride was administered as a suspension in corn oil by gavage, 5 days per week for 103 weeks.

Body Weights and Survival in the Two-Year Studies: Mean body weights of high dose rats were 5%-11% lower than those of vehicle controls during the second year of the studies. No significant differences in survival after 2 years were observed between any groups of rats of either sex (male: vehicle control, 36/60; low dose, 33/60; high dose, 32/60; female: 31/60; 27/60; 27/60). For mice, mean body weights of high dose males were generally 5%-12% lower than those of vehicle controls throughout the study.

Mean body weights of high dose female mice were 10%-32% lower than those of vehicle controls; mean body weights of low dose female mice were 10%-20% lower than those of vehicle controls. The survival of high dose male mice was significantly greater than that of vehicle controls after week 77 (survival after 2 years—male: 27/50; 30/50; 42/50; female: 37/50; 38/50; 41/50). No other differences in survival were observed between any groups of mice of either sex.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: At no site in rats or mice was there a chemical-related increase in the incidence of nonneoplastic or neoplastic lesions. A sufficient number of animals in each dose group lived long enough to allow evaluation of the potential carcinogenicity of succinic anhydride.

Genetic Toxicology: Succinic anhydride was not mutagenic in *S. typhimurium* with or without exogenous metabolic activation. The chemical did not induce sister chromatid exchanges or chromosomal aberrations in cultured CHO cells in the presence or absence of exogenous metabolic activation.

Conclusions: Under the conditions of these 2-year studies, there was *no evidence of carcinogenic activity* of succinic anhydride for male or female F344/N rats given 50 or 100 mg/kg succinic anhydride. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice given 38 or 75 mg/kg succinic anhydride or for female $B6C3F_1$ mice given 75 or 150 mg/kg.

Synonyms: butanedioic anhydride; dihydro-2,5-furandione; 2,5-diketotetrahydrofuran; succinic acid anhydride; succinyl anhydride; succinyl oxide; tetrahydro-2,5-dioxofuran

Report Date: January 1990

TR-374 Toxicology and Carcinogenesis Studies of Glycidol (CAS No. 556-52-5) In F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Glycidol is a viscous liquid that is used as a stabilizer in the manufacture of vinyl polymers, as an additive for oil and synthetic hydraulic fluids, and as a diluent in some epoxy resins. Toxicology and carcinogenesis studies were conducted by administering glycidol (94% pure, containing 1.2% 3-methoxy-1,2-propanediol, 0.4% 3-chloro-1,2-propanediol, 2.8% diglycidyl ether, and 1.1% 2,6-dimethanol-1,4-dioxane) in water by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary (CHO) cells, *Drosophila melanogaster*, and the bone marrow of male $B6C3F_1$ mice.

Sixteen-Day Studies: Glycidol doses for groups of five rats or five mice of each sex ranged from 37.5 to 600 mg/kg; vehicle controls received distilled water. All rats that received 600 mg/kg died between days 3 and 13. Edema and degeneration of the epididymal stroma, atrophy of the testis, and granulomatous inflammation of the epididymis occurred in males that received 300 mg/kg.

All mice that received 600 mg/kg and two males and two females that received 300 mg/kg died by day 4 of the studies. Focal demyelination in the medulla and thalamus of the brain occurred in all female mice that received 300 mg/kg.

Thirteen-Week Studies: Doses for groups of 10 rats ranged from 25 to 400 mg/kg, and doses for groups of 10 mice ranged from 19 to 300 mg/kg; vehicle controls received distilled water. All rats that received 400 mg/kg died by week 2; three males and one female that received 200 mg/kg died during weeks 11-12. Final mean body weights of male rats that received 50, 100, or 200 mg/kg were 96%-85% that of vehicle controls; final mean body weights of female rats receiving the same doses were 95%-89% that of vehicle controls. Sperm count and sperm motility were reduced in male rats that received 100 or 200 mg/kg. Necrosis of the cerebellum, demyelineation in the medulla of the brain, tubular degeneration and/or necrosis of the kidney, lymphoid necrosis of the thymus, and testicular atrophy and/or degeneration occurred in rats that received 400 mg/kg.

All mice that received 300 mg/kg died by week 2; deaths of mice that received 150 mg/kg occurred during weeks 4-8 for males and weeks 1-5 for females. Mean body weights of chemically exposed mice surviving to the end of the studies were generally 90%-94% those of vehicle controls. Sperm count and sperm motility were reduced in dosed male mice. Compound-related histopathologic lesions included demyelination of the brain in males and females that received 150 or 300 mg/kg, testicular atrophy in males at all doses, and renal tubular cell degeneration in male mice that received 300 mg/kg.

Based on reduced survival, reduced weight gain, and histopathologic lesions in the brain and kidney in rats that received 200 or 400 mg/kg and on reduced survival and histopathologic lesions of the brain in mice that received 150 or 300 mg/kg, doses selected for the 2-year studies of glycidol were 37.5 and 75 mg/kg for rats and 25 and 50 mg/kg for mice.

Body Weights and Survival in the Two-Year Studies: Mean body weights of chemically exposed male rats generally ranged from 80% to 94% of those of vehicle controls, and mean body weights of chemically exposed female rats were from 90% to 97% those of vehicle controls. Mean body weights of chemically exposed male mice were similar to those of vehicle controls; mean body weights of chemically exposed female mice were 79%-95% of those of vehicle controls. Virtually all male and female rats that received glycidol died or were killed in a moribund condition as a result of the early induction of neoplastic disease (final survival—male: vehicle control, 16/50; low dose, 0/50; high dose, 0/50; female: 28/50; 4/50; 0/50). Survival of vehicle control male rats was lower than that usually observed; however, specific causes of deaths could not be determined. The survival of male mice and low dose female mice was similar to that of vehicle controls; survival of female mice that received 50 mg/kg was lower than that of vehicle controls after week

101 (final survival – male: 33/50; 25/50; 27/50; female: 29/50; 27/50; 17/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Chemical-related nonneoplastic lesions in both rats and mice included hyperkeratosis and epithelial dysplasia of the forestomach. Fibrosis of the spleen was also present in rats of each sex, and cysts of the preputial gland and kidney were present in male mice.

Exposure to glycidol induced dose-related increases in the incidences of neoplasms in numerous tissues in both rats and mice (see summary table on page 5 of the Technical Report). In male rats, mesotheliomas arising in the tunica vaginalis and frequently metastasizing to the peritoneum were considered the major cause of early death. Early deaths in female rats were associated with the presence of mammary gland neoplasms.

Genetic Toxicology: Glycidol was mutagenic in a variety of in vitro and in vivo short-term tests. Mutagenic activity was observed in *S. typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 exposed to glycidol with and without exogenous metabolic activation. Glycidol was positive in the absence of exogenous metabolic activation in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y/TK cells; it was not tested with activation. In cytogenetic tests with CHO cells, glycidol induced both sister chromatid exchanges and chromosomal aberrations in the presence and absence of exogenous metabolic activation. Glycidol induced sex-linked recessive lethal mutations and reciprocal translocations in the germ cells of male *D. melanogaster* exposed by feeding. The incidence of micronucleated polychromatic erythrocytes was increased in the bone marrow of male $B6C3F_1$ mice administered glycidol by intraperitoneal injection.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of glycidol for male F344/N rats, based on increased incidences of mesotheliomas of the tunica vaginalis; fibroadenomas of the mammary gland; gliomas of the brain; and neoplasms of the forestomach, intestine, skin, Zymbal gland, and thyroid gland. There was *clear evidence of carcinogenic activity* for female F344/N rats, based on increased incidences of fibroadenomas and adenocarcinomas of the mammary gland; gliomas of the brain; neoplasms of the oral mucosa, forestomach, clitoral gland, and thyroid gland; and leukemia. There was *clear evidence of carcinogenic activity* for male $B6C3F_1$ mice based on increased incidences of neoplasms of the harderian gland, forestomach, skin, liver, and lung. There was *clear evidence of carcinogenic activity* for female $B6C3F_1$ mice, based on increased incidences of neoplasms of the harderian gland, mammary gland, uterus, subcutaneous tissue, and skin. Other neoplasms that may have been related to the administration of glycidol were fibrosarcomas of the glandular stomach in female rats and carcinomas of the urinary bladder and sarcomas of the epididymis in male mice.

Synonym: 2,3-epoxy-1-propanol

Report Date: March 1990

TR-375 Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers) (65%-71% *meta*-isomer and 32-35% *para*-isomer) (CAS No. 25013-15-4) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

Vinyl Toluene is used as a monomer in the plastics and surface-coating industries. Toxicology and carcinogenesis studies were conducted by exposing groups of F344/N rats and $B6C3F_1$ mice of each sex to vinyl toluene (mixed isomers: 65%-71% *meta* and 32%-35% *para*) by inhalation 6 hours per day, 5 days per week, for 15 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y cells, and Chinese hamster ovary (CHO) cells.

Fifteen-Day Studies: Rats were exposed to 0, 200, 400, 800, or 1,300 ppm vinyl toluene, and mice were exposed to 0, 10, 25, 50, 100, or 200 ppm. All rats lived to the end of the studies. The mean body weights at necropsy of rats exposed to 400-1,300 ppm were 13%-19% lower than that of controls for males and 9%-13% lower for females. Most male rats exposed to 1,300 ppm had centrilobular necrosis and focal inflammatory cell infiltration of the liver, whereas minimal centrilobular vacuolization of the liver was seen in all female rats exposed to 1,300 ppm. Dysplasia of the bronchial epithelial lining, chronic bronchitis, and lymphoid hyperplasia of the lung were observed in all rats exposed to 1,300 ppm.

Three of five male mice exposed to 200 ppm vinyl toluene died before the end of the studies. Four of five male mice exposed to 200 ppm had moderate-to-severe hepatocellular necrosis; all female mice exposed to 200 ppm had hyperplasia of the epithelium of the intrapulmonary bronchi and centrilobular necrosis, vacuolization, and inflammatory cell infiltrates in the liver.

Thirteen-Week Studies: Rats were exposed to 0, 25, 60, 160, 400, or 1,000 ppm vinyl toluene. All rats lived to the end of the studies. The final mean body weights of rats exposed to 400-1,000 ppm were 8%-19% lower than that of controls for males and 6%-12% lower for females. Relative liver weights for rats at 1,000 ppm were significantly greater than those for controls. The severity of nephropathy was increased in male rats exposed to 160, 400, or 1,000 ppm. Compound-related lesions were not observed in female rats.

Mice were exposed to 0, 10, 25, 60, or 160 ppm vinyl toluene. The final mean body weights of mice exposed to 25-160 ppm were 12%-20% lower than that of controls for males and 13%-16% lower for females. Inflammation of the lung was observed in 5/10 male and 3/9 female mice exposed to 160 ppm. Metaplasia of the nasal turbinates was seen in all exposed groups.

Based on these results, 2-year studies were conducted by exposing groups of 49 or 50 rats of each sex to 0, 100, or 300 ppm vinyl toluene by inhalation, 6 hours per day, 5 days per week for 103 weeks. Groups of 50 mice of each sex were exposed to 0, 10, or 25 ppm on the same schedule.

Body Weights and Survival in the Two-Year Studies: Mean body weights of male rats exposed to 300 ppm vinyl toluene and those of female rats exposed to 100 and 300 ppm were generally 4%-11% lower than those of controls. No significant differences in survival were seen between any groups of rats of either sex (male: control, 19/49; low dose, 17/50; high dose, 19/50; female: 31/50; 28/50; 26/50). Mean body weights of mice exposed to 25 ppm were 10%-23% lower than those of controls after week 8, whereas mice exposed to 10 ppm showed a weight decrement that was generally less than 10%. The survival of male mice exposed to 25 ppm was significantly greater than that of controls. No other significant differences in survival were seen between any groups of mice of either sex (male: 33/50; 30/50; 41/50; female: 36/50; 37/50; 34/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Degenerative and nonneoplastic proliferative lesions of the nasal mucosa were observed at increased incidences in exposed rats. These lesions included diffuse hyperplasia (goblet cell) of the respiratory epithelium with intraepithelial mucous cysts and focal erosion of the olfactory epithelium with cystic dilation (cysts) of the Bowman's glands. Focal respiratory epithelial metaplasia of the olfactory epithelium was seen in some exposed males, and cells with homogeneous eosinophilic cytoplasm in the olfactory epithelium occurred at increased incidences in exposed female rats. Neoplasms of the nasal mucosa were not seen in male or female rats.

There were no chemically related increases in neoplasm incidence in exposed male or female rats.

Degenerative and inflammatory lesions of the nasal mucosa were observed at increased incidences in exposed mice. These lesions included focal chronic active inflammation and diffuse hyperplasia of the respiratory epithelium. Chronic active inflammation of the bronchioles occurred in many exposed mice but not in controls. Neoplasms of the nasal passage were not observed in mice.

There were no chemically related increases in neoplasm incidence in exposed male or female mice. Exposure-related decreased incidences included alveolar/bronchiolar neoplasms (control, 12/50; 10 ppm, 5/49; 25 ppm, 2/49) and malignant lymphomas (7/50; 3/50; 0/50) in males and hepatocellular neoplasms (9/48; 5/16; 2/49) in females.

Genetic Toxicology: Vinyl toluene did not induce gene mutations in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation (S9). Vinyl toluene was positive in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y/TK cells in the absence of S9; it was not tested with S9. Vinyl toluene did not induce sister chromatid exchanges or chromosomal aberrations in CHO cells with or without S9.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity* for male or female F344/N rats exposed to 100 or 300 ppm vinyl toluene and *no evidence of carcinogenic activity* for male or female B6C3F$_1$ mice exposed to 10 or 25 ppm.

There was evidence of chemical-related toxicity to the nasal passage in both rats and mice.

Synonyms: 3-vinyl toluene and 4-vinyl toluene (mixed isomers)

Report Date: March 1990

TR-376 Toxicology and Carcinogenesis Studies of Allyl Glycidyl Ether (CAS No. 106-92-3) in Osborne-Mendel Rats and B6C3F$_1$ Mice (Inhalation Studies)

Allyl glycidyl ether is used as a resin intermediate and as a stabilizer of chlorinated compounds, vinyl resins, and rubber. Toxicology and carcinogenesis studies were conducted by exposing groups of Osborne-Mendel rats and B6C3F$_1$ of each sex to allyl gylcidyl ether (greater than 97% pure) by inhalation for 6 hours per day, 5 days per week for 2 weeks, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary (CHO) cells, and *Drosophila melanogaster*. Studies of reproductive effects were conducted in rats and mice exposed to allyl glycidyl ether for 8 weeks.

Two-Week Studies: Exposure concentrations ranged up to 500 ppm in rats and 100 ppm in mice. All rats that were exposed to 500 ppm died; no deaths occurred at the next lower (200 ppm) exposure concentration. All male mice and 3/5 female mice exposed to 100 ppm and 2/5 male mice and 1/5 female mice exposed to 50 ppm died. Compound-related lesions in rats and mice included acute inflammation of the nasal passage and major airways.

Eight-Week Studies of Reproductive Effects: Rats were exposed to 0-200 ppm allyl glycidyl ether, and mice were exposed to 0-30 ppm, 6 hours per day, 5 days per week for 8 weeks. The mating performance of exposed male rats was markedly reduced; however, sperm motility and number were not affected. No deficiencies were seen in the reproductive performance of exposed female rats or male or female mice.

Thirteen-Week Studies: Exposure concentrations ranged up to 200 ppm for rats and 30 ppm for mice. All rats lived to the end of the studies. The final mean body weights of male rats exposed to 10-200 ppm were 7%-24% lower than that of controls. Clinical signs attributable to irritation of the upper respiratory tract and eyes were seen in exposed animals. Histologic lesions included squamous metaplasia of the nasal passage in all exposure groups (4 ppm, lowest concentration) and involved both the respiratory epithelium and the olfactory epithelium. The lesions were more severe anteriorly and dorsally and with increasing concentration. At 30 ppm and higher, erosion was seen in the nasal passage and squamous metaplasia was seen in the upper airways.

There were no compound-related deaths in mice. The final mean body weights of mice exposed to 30 ppm were 12% lower than those of controls for both males and females. Mice exposed to 10 or 30 ppm allyl glycidyl ether

had squamous metaplasia of the nasal passage, involving both the respiratory epithelium and the olfactory epithelium, which tended to be more severe in the anterior and dorsal portions of the nasal passage. In mice exposed to 30 ppm, epithelial erosions were also found.

Body Weights and Survival in the Two-Year Studies: Two-year studies were conducted by exposing groups of 50 Osborne-Mendel rats and B6C3F$_1$ mice of each sex to 0, 5, or 10 ppm allyl glycidyl ether by inhalation for 6 hours per day, 5 days per week for 102 or 103 weeks. Mean body weights of the exposed rats were within 8% of those of controls throughout the studies. Mean body weights of mice exposed to 5 or 10 ppm were 5%-20% lower than those of controls. Deaths were seen in all groups of male rats beginning at 1 year of age (final survival – control, 12/50; 5 ppm, 11/50; 10 ppm, 8/50). Survival of female rats was not exposure related (24/50; 30/50; 25/50). Exposed mice had slightly increased survival (male mice: 38/50; 39/50; 46/50; female mice: 33/50; 42/50; 41/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: In male rats exposed to 10 ppm allyl glycidyl ether, three apparently unrelated neoplasms of the nasal passage were found. Two neoplasms, a papillary adenoma and a squamous cell carcinoma, appeared to arise from different cell types in the respiratory epithelium. One poorly differentiated adenocarcinoma in the olfactory region was also found. One papillary adenoma of respiratory epithelial origin was found in a female rat exposed to 5 ppm. Exposure-related nonneoplastic lesions of the nasal passages in rats included inflammation, squamous metaplasia, respiratory metaplasia (replacement of olfactory epithelium by ciliated epithelium), hyperplasia of the respiratory epithelium, and degeneration of the olfactory epithelium. In male mice exposed to 10 ppm allyl glycidyl ether, a hemangioma and three papillary adenomas were present in the nasal passage. In female mice exposed to 10 ppm, a hemangioma and an adenoma were found in the nasal passage. Nonneoplastic lesions of the nasal passages in mice included inflammation, squamous metaplasia, hyperplasia, basal cell hyperplasia, dysplasia of the respiratory epithelium, and metaplasia of the olfactory epithelium. In male mice, there was an exposure-related decrease in the incidences of hepatocellular neoplasms; in female mice, there was a decrease in the incidences of pituitary gland adenomas.

Genetic Toxicology: Allyl glycidyl ether was mutagenic in *S. typhimurium* strains TA100 and TA1535 with and without exogenous metabolic activation; no mutagenic activity was observed in strains TA98 or TA1537. Allyl glycidyl ether induced sister chromatid exchanges and chromosomal aberrations in CHO cells both in the presence and the absence of metabolic activation. A significant increase in sex-linked recessive lethal mutations was recorded in the germ cells of male *D. melanogaster* fed a sucrose solution containing allyl glycidyl ether, but no increase in reciprocal translocations occurred in these cells.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *equivocal evidence of carcinogenic activity* of allyl glycidyl ether for male Osborne-Mendel rats, based on the presence of one papillary adenoma of respiratory epithelial origin, one squamous cell carcinoma of respiratory epithelial origin, and one poorly differentiated adenocarcinoma of olfactory epithelial origin, all occurring in the nasal passage of males exposed to 10 ppm. There was *no evidence of carcinogenic activity* of allyl glycidyl ether for female rats. One papillary adenoma of the respiratory epithelium was present in a female rat exposed to 5 ppm. There was *some evidence of carcinogenic activity* of allyl glycidyl ether for male B6C3F$_1$ mice, based on the presence of three adenomas of the respiratory epithelium, dysplasia in four males, and focal basal cell hyperplasia of the respiratory epithelium in seven males in the nasal passage of mice exposed to 10 ppm. There was *equivocal evidence of carcinogenic activity* of allyl glycidyl ether for female mice, based on the presence of one adenoma of the respiratory epithelium and focal basal cell hyperplasia of the respiratory epithelium in seven females exposed to 10 ppm. The sensitivity of the assay to detect potential carcinogenicity may have been reduced in male rats because of poor survival in all groups.

In exposed mice, body weights were decreased 10% or more, mortality was decreased, and there were lower incidences of liver neoplasms (males) and pituitary gland adenomas (females) compared with controls.

Significant exposure-related nonneoplastic lesions were restricted to the nasal passage in both rats and mice and induced inflammation, metaplasia, respiratory epithelial hyperplasia, and olfactory epithelial degeneration. Basal cell hyperplasia and dysplasia of the respiratory epithelium of the nasal passage were found only in the mice.

Synonyms: allyl 2,3-epoxypropyl ether; 1-allyloxy-2,3-epoxypropane; 1,2-epoxy-3-allyloxypropane; glycidyl allyl ether; ((2-propenyloxy)methyl)oxirane; 1-(allyloxy)-2,3-epoxypropane

Report Date: January 1990

TR-377 Toxicology and Carcinogenesis Studies of CS2 (94% *o*-Chlorobenzalmalononitrile, CAS No. 2698-41-1) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

CS2 (94% *o*-chlorobenzalmalononitrile [CS]; 5% Cab-O-Sil® colloidal silica; 1% hexamethyldisilizane), an eye and respiratory irritant, is used as an aerosol to control riots. Toxicology and carcinogenesis studies were conducted by exposing groups of F344/N rats and B6C3F$_1$ mice of each sex for 6 hours per day, 5 days per week for 2 weeks, 13 weeks, or 2 years, to a CS2 aerosol. Genetic toxicology studies with CS2 were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: At exposure concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 CS2, all rats exposed to 30 or

100 mg/m^3 and all mice exposed to 10, 30, or 100 mg/m^3 died before the end of the studies. Compound-related clinical signs observed included erythema, blepharospasm, listlessness, nasal discharge, and mouse breathing.

Thirteen-Week Studies: At exposure concentrations of 0, 0.4, 0.75, 1.5, 3, or 6 mg/m^3, 1/10 male rats exposed to 6 mg/m^3 died before the end of the studies. Final mean body weights of rats exposed to 1.5 mg/m^3 or more were 17%-44% lower than that of controls for males and 10%-24% lower for females. The absolute and relative thymus weights were reduced for exposed male and female rats, particularly at 6 mg/m^3. Compound-related lesions of the nasal passage in rats included focal erosion with regenerative hyperplasia and squamous metaplasia of the respiratory epithelium and suppurative inflammation. Acute inflammation and hyperplasia of the respiratory epithelium were seen in the larynx and trachea of some exposed rats.

All mice exposed to 6 mg/m^3 and 1/10 males and 1/10 females exposed to 3 mg/m^3 died before the end of the studies. Final mean body weights of mice exposed to 3 mg/m^3 were 13% lower than that of controls for males and 9% lower for females. Compound-related lesions of the nasal passage in mice included squamous metaplasia of the nasal respiratory epithelium and inflammation.

Based on these results, CS2 exposure concentrations for the 2-year studies were 0, 0.075, 0.25, or 0.75 mg/m^3 for 6 hours per day, 5 days per week for 105 weeks for groups of 50 rats of each sex. Groups of 50 mice of each sex were exposed to 0, 0.75, or 1.5 mg/m^3 on the same schedule.

Body Weights and Survival in the Two-Year Studies: Final mean body weights of rats exposed to 0.75 mg/m^3 were 7%-11% lower than those of controls. Final mean body weights of mice exposed to CS2 were lower than those of controls (male: 5% and 9%; female: 10% and 17%). No compound-related clinical signs were observed. No significant differences in survival were seen for any group of rats or mice of either sex.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Compound-related nonneoplastic lesions occurred in the nasal passage of exposed rats and mice. In exposed rats, hyperplasia and squamous metaplasia of the respiratory epithelium and degeneration of the olfactory epithelium with ciliated columnar and/or squamous metaplasia were observed. Focal chronic inflammation and proliferation of the periosteum of the turbinate bones were increased slightly in rats at the top exposure concentration. Suppurative inflammation with hyperplasia and squamous metaplasia of the respiratory epithelium occurred in exposed mice.

There were no compound-related increased incidences of neoplasms in rats or mice exposed to CS2. In exposed female mice, there were pronounced decreases in the incidences of adenomas of the pituitary pars distalis (control, 13/47; 0.75 mg/m^3, 5/46; 1.5 mg/m^3, 1/46) and decreased incidences of malignant lymphomas (21/50; 12/50; 8/50).

Genetic Toxicology: The responses in Salmonella gene mutation tests with CS2 were equivocal in one laboratory for strain TA100 in the absence of exogenous metabolic activation (S9) and equivocal in another laboratory for TA97 with S9; in all other strains tested, CS2 was clearly negative with or without S9. CS2 induced trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells in the absence of S9; it was not tested with S9. CS2 induced both sister chromatid exchanges and chromosomal aberrations in CHO cells with and without S9.

Conclusions: Under the conditions of these inhalation studies, there was *no evidence of carcinogenic activity* of CS2 for male or female F344/N rats exposed to 0.075, 0.25, or 0.75 mg/m^3 in air for up to 2 years. There was *no evidence of carcinogenic activity* for male or female B6C3F$_1$ mice exposed to 0.75 or 1.5 mg/m^3 in air for up to 2 years. Concentration-related decreases in the incidences of pituitary gland adenomas and lymphomas were observed in female mice.

Exposure to CS2 caused degeneration and squamous metaplasia of the olfactory epithelium, hyperplasia and metaplasia of the respiratory epithelium, and proliferation of the periosteum of the nasal passage of rats. In mice, exposure to this compound caused suppurative inflammation and hyperplasia and squamous metaplasia of the respiratory epithelium of the nasal passage.

Report Date: March 1990

TR-378 Toxicology and Carcinogenesis Studies of Benzaldehyde (CAS No. 100-52-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Benzaldehyde is an aromatic aldehyde used in the food, beverage, pharmaceutical, perfume, soap, and dyestuff industries. Toxicology and carcinogenesis studies were conducted by administering benzaldehyde (99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, Chinese hamster ovary (CHO) cells, and *Drosophila melanogaster*.

Sixteen-Day Studies: All rats that received 1,600 mg/kg died by day 2, and 2/5 males and 2/5 females that received 800 mg/kg died before the end of the studies. Final mean body weights of dosed and vehicle control rats were similar, with the exception of the 800 mg/kg groups, in which males were 14% lighter and females were 11% lighter than vehicle controls. All mice that received 1,600 or 3,200 mg/kg died by day 3. Final mean body weights of dosed and vehicle control mice were similar. No gross lesions attributable to benzaldehyde were detected upon necropsy.

Thirteen-Week Studies: Six of 10 male rats and 3/10 female rats that received 800 mg/kg and 1/10 female rats that received 400 mg/kg died near the end of the studies. Final mean body weights of dosed and vehicle control

rats were similar, with the exception of male rats receiving 800 mg/kg, which were 26% lighter than vehicle controls. Compound-related lesions seen in rats receiving 800 mg/kg, but not in those receiving 400 mg/kg, included degeneration and necrosis in the cerebellum, necrosis in the hippocampus, hyperplasia and/or hyperkeratosis in the forestomach, and degeneration or necrosis of the liver and of the tubular epithelium in the kidney.

Nine of 10 male mice and 1/10 female mice that received 1,200 mg/kg benzaldehyde died by the end of the first week. Compound-related renal tubule degeneration and/or necrosis and reduction in final body weight were observed in the 600 mg/kg group of male mice. No reductions in body weight or compound-related lesions were seen in female mice.

Based on observations of compound-related lesions involving the brain, forestomach, kidney, and liver of male and female rats and the kidney of male mice in the 13-week studies, 2-year studies were conducted by administering 0, 200, or 400 mg/kg benzaldehyde in corn oil by gavage, 5 days per week for 103 weeks to groups of 50 male and 50 female rats and for 104 weeks to groups of 50 male mice. Based on survival data from the 16-day and 13-week studies, groups of 50 female mice were administered 0, 300, or 600 mg/kg benzaldehyde for 103 weeks.

Body Weights and Survival in the Two-Year Studies: Mean body weights of dosed rats and mice were similar to their respective vehicle controls throughout the studies. The survival of the high dose group of male rats was lower than that of the vehicle controls after 1 year; no other significant differences were observed between any groups of rats or mice (survival—male rats; vehicle control, 37/50; low dose, 29/50; high dose, 21/50; female rats: 33/50; 33/50; 29/50; male mice: 32/50; 33/50; 31/50, female mice: 30/50; 27/50; 35/50).

Nonneoplastic and Neoplastic Effects in the Two-year Studies: The only effects of benzaldehyde were those seen in the forestomach of mice. The incidences of uncommonly occurring squamous cell papillomas of the forestomach in both exposure groups were significantly greater than those in vehicle controls (male: vehicle control, 1/50; low dose, 2/50; high dose, 5/50; female: 0/50; 5/50; 6/50). The increased incidences of papillomas were accompanied by dose-related increases in the incidences in forestomach hyperplasia (male: 7/50; 8/50; 16/50; female: 12/50; 23/50; 39/50).

Genetic Toxicology: Benzaldehyde was not mutagenic in six strains of *S. typhimurium* and did not induce chromosomal aberrations in CHO cells, with or without exogenous metabolic activation. Benzaldehyde induced increases in trifluorothymidine-resistant mouse lymphoma cells in the absence exogenous metabolic activation and increased sister chromatid exchanges in CHO cells in both the presence and absence of metabolic activation. Sex-linked recessive lethal mutations were not induced in the germ cells of adult male *D. melanogaster* administered benzaldehyde by feeding or by injection.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of benzaldehyde for male or female F344/N rats receiving 200 or 400 mg/kg per day. There was *some evidence of carcinogenic activity* of benzaldehyde for male or female $B6C3F_1$ mice, as indicated by increased incidences of squamous cell papillomas and hyperplasia of the forestomach. Female rats and male and female mice might have been able to tolerate higher doses.

Synonyms: artificial almond oil; artificial essential oil of almond; benzenecarbonal; benzene carbaldehyde; benzoic aldehyde; phenylmethanal

Report Date: March 1990

TR-379 Toxicology and Carcinogenesis Studies of 2-Chloroacetophenone (CAS No. 532-27-4) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

2-Chloroacetophenone is a potent lacrimator that has been used as a riot control agent and in tear gas formulations for personal protection devices. Toxicology and carcinogenesis studies were conducted by exposing groups of F344/N rats and $B6C3F_1$ mice of each sex to air containing 2-chloroacetophenone vapor for 14 days, 13 weeks, 15 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: In 14-day studies, exposure concentrations of 2-chloroacetophenone ranged from 4.8 to 64 mg/m^3. All rats exposed to 19, 43, or 64 mg/m^3 died during the first week of the studies and 1/5 male rats exposed to 10 mg/m^3 died during the second week of the study. Rats exposed to 10 mg/m^3 lost weight; the final mean body weights of male or female rats exposed to 4.8 mg/m^3, the lowest concentration used, were 23% or 15% lower than that of controls. During the exposure, rats showed partial closure of the eyelids, excessive lacrimation (dacryorrhea), dyspnea, and erythema. All mice exposed to 10 mg/m^3 or higher concentrations of 2-chloroacetophenone died during the first week of the studies. The final mean body weights of mice exposed to 4.8 mg/m^3 were similar to those of controls. Dacryorrhea was observed in exposed mice.

Thirteen-Week Studies: The exposure concentrations of 2-chloroacetophenone ranged from 0.25 to 4 mg/m^3 for rats and mice. All rats lived to the end of the studies. The final mean body weights of rats exposed to 4 mg/m^3 were 9% lower than those of controls. Eye irritation during exposure was evident in rats exposed to 0.5 mg/m^3 or higher concentrations of 2-chloroacetophenone. One of 10 female mice exposed to 4 mg/m^3 and 1/10 female mice exposed to 0.5 mg/m^3 died before the end of the study. The final mean body weights of exposed mice were 7%-12% lower than that of controls for males and 12%-15% lower for females. No chemical-related gross or microscopic lesions were observed in rats or mice.

In the 2-year studies, groups of 60 rats of each sex were exposed to a vapor of 0 (chamber control), 1, or 2 mg/m^3 (0, 0.15, or 0.3 ppm) 2-chloroacetophenone, 6 hours per

day, 5 days per week. Groups of 60 mice of each sex were exposed to 0 (chamber control), 2, or 4 mg/m^3 (0, 0.3, or 0.6 ppm) on the same schedule. Ten animals from each group were killed and examined at 15 months; the remaining animals continued on study for 2 years.

Fifteen-Month Studies: In the 15-month studies, minimal-to-mild focal squamous metaplasia and hyperplasia of the respiratory epithelium were seen at increased incidences in rats exposed to 2 mg/m^3. No exposure-related lesions were observed in mice of either sex.

Body Weight and Survival in the Two-Year Studies: Mean body weights and survival of exposed and chamber control rats were similar throughout most of the studies (survival-male: control, 14/50; 1 mg/m^3, 22/50; 2 mg/m^3, 17/50; female: 23/50; 20/50; 24/50). Mean body weights of male mice exposed to 4 mg/m^3 were about 5%-12% lower than those of controls after week 30; small differences between mean body weights of exposed and control female mice were not clearly exposure related. The survival of female mice exposed to 2 mg/m^3 was significantly lower than that of chamber controls after week 98. No other differences in survival were observed between any groups of mice (male: control, 34/50; 2 mg/m^3, 36/50; 4 mg/m^3, 33/50; female: 40/50; 28/50; 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Fibroadenomas of the mammary gland occurred in female rats with positive trends, and the incidence in the 2 mg/m^3 group was greater than that in chamber controls (control, 12/50; 1 mg/m^3, 19/50; 2 mg/m^3, 23/50). The incidences of adenomas or adenocarcinomas of the mammary gland were not increased in the exposed groups.

Minimal-to-mild suppurative inflammation of the nasal mucosa was observed at increased incidences in exposed male rats. Hyperplasia and squamous metaplasia of the nasal respiratory epithelium were observed at increased incidences in exposed male and female rats. In mice, squamous metaplasia of the respiratory epithelium of the nasal passage was seen in four females and two males exposed to 4 mg/m^3 2-chloroacetophenone.

Inflammation, ulcers, and squamous hyperplasia of the forestomach were observed at increased incidences in exposed female rats.

Genetic Toxicology: 2-Chloroacetophenone was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation. In cytogenetic tests with CHO cells, 2-chloroacetophenone did not induce sister chromatid exchanges with or without activation, but a weak positive increase in chromosomal aberrations was observed in the absence of metabolic activation.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity* of 2-chloroacetophenone for male rats exposed to 1 or 2 mg/m^3. There was *equivocal evidence of carcinogenic activity* for female F344/N rats, based on a marginal increase in fibroadenomas of the mammary gland. There was *no evidence of carcinogenic activity* for male or female B6C3F$_1$ mice exposed to 2 or 4 mg/m^3 2-chloroacetophenone.

Synonyms: α-chloroacetophenone; 2-chloro-1-phenylethanone; CN; phenacyl chloride; phenylchloromethylketone

Trade Names: Mace®; Chemical Mace®

Report Date: March 1990

TR-380 Toxicology and Carcinogenesis Studies of *l*-Epinephrine Hydrochloride (CAS No. 55-31-2) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

l-Epinephrine, an endogenous neurotransmitter hormone, is widely used for the treatment of allergic and respiratory disorders. Toxicology and carcinogenesis studies of epinephrine hydrochloride were conducted by exposing groups of F344/N rats and B6C3F$_1$ mice of each sex to an aerosol containing epinephrine hydrochloride for 14 days, 13 weeks, 15 months, or 2 years. During the 14-day and 13-week studies, control animals were exposed to dilute aerosols of hydrochloric acid (pH 2.8), whereas during the 15-month and 2-year studies, controls were exposed to aerosols of water. Genetic toxicology studies of epinephrine were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: Rats and mice were exposed to 0 or 12.5-200 mg/m^3 epinephrine hydrochloride. Deaths occurred in male rats exposed to 12.5 mg/m^3 or more and in females exposed to 25 mg/m^3 or more. Deaths of mice occurred at concentrations of 50 mg/m^3 or higher. Compound-related clinical signs included an increased respiratory rate in all groups of epinephrine-exposed rats and mice. At higher concentrations (100 and 200 mg/m^3), excessive lacrimation and dyspnea in rats and exaggerated visual and auditory reflexes in mice were observed.

Thirteen-Week Studies: Rats and mice were exposed to 0 or 2.5-40 mg/m^3 epinephrine hydrochloride. Deaths in rats and mice were not concentration related. Final mean body weights of chemically exposed and hydrochloric acid aerosol control rats and mice were generally similar. Increased respiratory rates were noted in rats and mice exposed to 40 mg/m^3. Heart and adrenal gland weights of rats and mice and liver weights of mice exposed to 40 mg/m^3 were greater than those of aerosol controls. Squamous metaplasia occurred in the respiratory epithelium of the nasal mucosa of rats and mice exposed to 40 mg/m^3. Degenerative lesions of the laryngeal muscle were seen in male and female rats exposed to 20 or 40 mg/m^3. Inflammation in the glandular stomach was seen in male and female mice exposed to 10, 20, and 40 mg/m^3, and uterine atrophy was seen in 7/10 female mice exposed to 40 mg/m^3.

Two-year studies were conducted by exposing groups of 60 rats or each sex to 0, 1.5, or 5 mg/m^3 epinephrine hydrochloride, 5 days per week for 103 weeks. Groups of 60 mice of each sex were exposed to 0, 1.5, or 3 mg/m^3

epinephrine hydrochloride, 5 days per week for 104 weeks. Use of these exposure concentrations represented a departure from the usual practice of utilizing doses equivalent to one-half the maximum tolerated dose (MTD) and the MTD for 2-year carcinogenicity studies. Thus, although the dose levels exceeded maximum human therapeutic use levels (normalized to body weight and surface area), they were less than one-half the MTD.

Fifteen-Month Studies: Results of hematologic analyses did not show compound-related changes. Absolute liver weights for exposed mice (3 mg/m^3) and rats (5 mg/m^3) and relative liver weights for exposed rats (5 mg/m^3) were significantly lower than those for controls. The absolute kidney weights for mice exposed to 3 mg/m^3 and the kidney weight to body weight ratio for male mice exposed to 3 mg/m^3 were significantly lower than those for controls. No compound-related lesions were seen in rats or mice.

Body Weights and Survival in the Two-Year Studies: Mean body weights and survival of exposed and control rats and mice were similar (survival, rats – male: control, 33/50; 1.5 mg/m^3, 27/50; 5 mg/m^3, 32/50; female: 32/50; 29/50; 30/50; mice – male: control, 33/50; 1.5 mg/m^3, 34/50; 3 mg/m^3, 36/50; female: 32/50; 35/50; 34/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Suppurative inflammation of the nasal mucosa, dilatation of the nasal glands (Bowman's and septal), and hyperplasia of the respiratory epithelium were seen at increased incidences in male rats exposed to 5 mg/m^3 and in female rats exposed to 1.5 or 5 mg/m^3.

Hyaline degeneration of the olfactory epithelium in male mice and suppurative inflammation of the nasal passage and hyaline degeneration of the respiratory epithelium in female mice were increased in the 1.5 and 3 mg/m^3 groups compared with controls. No neoplasms seen in these studies were considered related to chemical exposure.

Genetic Toxicology: Salmonella gene mutation tests with *l*-epinephrine yielded negative results in strain TA100 in the presence of exogenous metabolic activation (S9) and equivocal results in the absence of S9. No mutagenic activity was observed in strains TA98, TA1535, or TA1537 with or without S9. The responses observed in the CHO cell assay for induction of sister chromatid exchanges were considered to be negative and equivocal in the presence and absence of S9 activation, respectively. *l*-Epinephrine did not induce chromosomal aberrations in CHO cells with or without S9.

Conclusions: Under the conditions of these 2-year studies, no carcinogenic effects were observed in male or female F344/N rats exposed to aerosols containing 1.5 or 5 mg/m^3 *l*-epinephrine hydrochloride for 2 years or in B6C3F$_1$ mice exposed to 1.5 or 3 mg/m^3 *l*-epinephrine hydrochloride for 2 years. However, these studies were considered to be *inadequate studies of carcinogenic activity* because the concentrations used, which were chosen to represent multiples of therapeutic doses, were considered too low for the animals to have received an adequate systemic challenge from the compound.

Synonyms: adrenaline hydrochloride; 4-(1-hydroxy-2-(methylamino)ethyl)-1,2-benzenediol; (-)3,4-dihydroxy-α-((methylamino)methyl)benzyl alcohol hydrochloride; methylaminoethanol catechol hydrochloride

Trade names for epinephrine formulations: Primatene® Mist; Sus-Phrine®; Epipen®; Supravenin Hydrochloride®; Bronkaid®

Report Date: March 1990

TR-381 Toxicology and Carcinogenesis Studies of *d*-Carvone (CAS No. 2244-16-8) in B6C3F$_1$ Mice (Gavage Studies)

d-Carvone occurs naturally in caraway and dill seeds and in many essential oils; it has been used as a carminative and in perfumes and soaps. Toxicity and carcinogenesis studies were conducted by administering *d*-carvone (approximately 96% pure) in corn oil by gavage to groups of male and female B6C3F$_1$ mice for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Sixteen-Day Studies: All mice that received 1,600 or 3,500 mg/kg died within 7 days. Relative liver weights were increased for dosed male mice, and relative thymus weights were decreased for dosed female mice. No compound-related lesions were observed.

Thirteen-Week Studies: All male mice and 9/10 female mice that received the top dose of 1,500 mg/kg died before the end of the studies. No compound-related histopathologic changes were observed.

Based on survival at the high doses in the 13-week studies, 2-year toxicology and carcinogenesis studies were conducted by administering *d*-carvone in corn oil by gavage to groups of 50 male and 50 female mice at doses of 375 or 750 mg/kg, 5 days per week for 103 weeks.

Two-Year Studies: Mean body weights of dosed and vehicle control mice were similar throughout the studies. Survival of dosed male mice was similar to that of vehicle controls (vehicle control, 37/50; low dose, 42/50; high dose, 36/50); survival of dosed female mice was greater than that of vehicle control female mice (14/50; 29/50; 38/50). Apparently, abscesses in the urogenital system caused the early deaths of many vehicle control female mice.

No neoplastic lesions attributed to *d*-carvone dosing were observed in mice.

Genetic Toxicology: *d*-Carvone was not mutagenic in *S. typhimurium* but induced sister chromatid exchanges and chromosomal aberrations in CHO cells.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of *d*-carvone for male or female B6C3F$_1$ mice administered 375 or 750 mg/kg, 5 days per week for 2 years.

Synonyms for *d*-carvone: (+)-carvone; *d*(+)-carvone; (*S*)-carvone; (*S*)-(+)carvone; (*S*)-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one; (*S*)-*d*-*p*-mentha-6-8,(9)-dien-2-one; (*S*)-(+)-*p*-mentha-6,8-dien-2-one; *d*-1-methyl-4-isopropenyl-6-cyclohexen-2-one. Carvol is a synonym for carvone (*d*, *l* not specified)

Report Date: February 1990

TR-382 Toxicology and Carcinogenesis Studies of Furfural (CAS No. 98-01-1) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Furfural is used as a precursor for the manufacture of furan, furfuryl alcohol, tetrahydrofuran, and their derivatives and as an industrial solvent. Furfural is also present in numerous processed food and beverage products. Toxicology and carcinogenesis studies were conducted by administering furfural (99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, Chinese hamster ovary (CHO) cells, *Drosophila melanogaster*, and mouse bone marrow cells.

Sixteen-Day Studies: Rats received doses ranging from 15 to 240 mg/kg, and mice received doses from 25 to 400 mg/kg. Eight of 10 rats that received 240 mg/kg died within 3 days. Final mean body weights of chemically exposed animals were similar to those of vehicle controls; no compound-related histologic lesions were observed in any dosed groups.

Thirteen-Week Studies: Rats received doses ranging from 11 to 180 mg/kg, and mice received doses from 75 to 1,200 mg/kg. Most rats that received 180 mg/kg died; mean body weights of chemically exposed rats were similar to those of vehicle controls. Mean relative and absolute liver and kidney weights were increased in male rats that received 90 mg/kg, and cytoplasmic vacuolization of hepatocytes was increased in chemically exposed male rats.

Almost all mice that received doses of 600 or 1,200 mg/kg died within the first 3 weeks. Mean body weights of chemically exposed mice were similar to those of vehicle controls throughout the studies. Mean absolute liver weights and liver weight to body weight ratios were increased in females that received 300 mg/kg. Centrilobular coagulative necrosis and/or multifocal subchronic inflammation of the liver were present in chemically exposed mice but not in vehicle control mice.

Based on these results, doses selected for the 2-year studies were 0, 30, and 60 mg/kg for rats and 0, 50, 100, and 175 mg/kg for mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of chemically exposed and vehicle control animals were similar throughout the studies for rats and mice. Two-year survival of male rats; low dose female rats, and mice was unaffected by chemical exposure (male rats: vehicle control, 31/50; low dose, 28/50; high dose, 24/50; female rats: 28/50; 32/50; 18/50; male mice: vehicle control, 35/50; low dose, 28/50; mid dose, 24/50; high dose, 27/50; female mice: 33/50; 28/50; 29/50; 32/50). Survival of high dose female rats was reduced by deaths associated with gavage administration; the administration of furfural was considered to be a contributing factor in these gavage-related deaths.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Centrilobular necrosis of the liver occurred at increased incidences in chemically exposed male rats (vehicle control, 3/50; low dose, 9/50; high dose, 12/50). Two high dose male rats had bile duct dysplasia with fibrosis, and two had cholangiocarcinomas; neither lesion was seen in the other dose groups. The historical incidence of bile duct neoplasms in corn oil vehicle control male rats is 3/2,145 (0.1%).

Multifocal pigmentation and chronic inflammation of the subserosa of the liver occurred in chemically exposed mice (pigmentation – male: 0/50; 0/50; 8/49; 18/50; female: 0/50; 0/50; 0/50; 11/50; chronic inflammation – male: 0/50; 0/50; 8/49; 18/50; female: 0/50; 0/50; 1/50; 8/50). The incidences of hepatocellular adenomas and hepatocellular carcinomas in male mice and hepatocellular adenomas in female mice were significantly increased in the high dose group compared with those in the vehicle controls (male – adenomas: 9/50; 13/50; 11/49; 19/50; carcinomas: 7/50; 12/50; 6/49; 21/50; female – adenomas: 1/50; 3/50; 5/50; 8/50; adenomas or carcinomas, combined: 5/50; 3/50; 7/50; 12/50).

Three renal cortical adenomas or carcinomas occurred in chemically exposed male mice (0/50; 1/50; 1/49; 1/50), and a renal cortical adenoma was present in one low dose female mouse; the historical incidence of renal cortical neoplasms in National Toxicology Program 2-year corn oil gavage studies in male B6C3F$_1$ mice is 8/2,183.

Forestomach hyperplasia occurred in chemically exposed female mice, and squamous cell papillomas were increased in high dose female mice (hyperplasia: 0/50; 5/50; 5/50; 3/50; papillomas: 1/50; 0/50; 1/50; 6/50).

Genetic Toxicology: In gene mutation tests with four strains of Salmonella (TA98, TA100, TA1535, and TA1537), no mutagenic activity was observed in the presence or absence of exogenous metabolic activation (S9) in one laboratory and an equivocal response was observed in TA100 in the absence of S9 in a second laboratory. Exposure to furfural induced trifluorothymidine resistance in mouse L5178Y lymphoma cells in the absence of S9 (no evaluation was made in the presence of S9), sister chromatid exchanges (SCEs) and chromosomal aberrations in CHO cells in the presence or absence of S9, and an increase in sex-linked recessive lethal mutations but no reciprocal translocations in germ cells of *D. melanogaster*; furfural did not induce SCEs or chromosomal aberrations in the bone marrow of B6C3F$_1$ mice.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of furfural for male F344/N rats based on

the occurrence of uncommon cholangiocarcinomas in two animals and bile duct dysplasia with fibrosis in two other animals. There was *no evidence of carcinogenic activity* for female F344/N rats that received doses of 0, 30, or 60 mg/kg furfural. There was *clear evidence of carcinogenic activity* for male $B6C3F_1$ mice, based on increased incidences of hepatocellular adenomas and hepatocellular carcinomas. There was *some evidence of carcinogenic activity* in female $B6C3F_1$ mice, based on increased incidences of hepatocellular adenomas. Renal cortical adenomas or carcinomas in male mice and squamous cell papillomas of the forestomach in female mice may have been related to exposure to furfural.

Synonyms: 2-furancarboxaldehyde; 2-furaldehyde; pyromucic aldehyde

Common Name: Artificial oil of ants

Report Date: March 1990

TR-383 Toxicology and Carcinogenesis Studies of 1-Amino-2,4-Dibromoanthraquinone (CAS: 81-49-2) in F344 Rats and $B6C3F_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-384 Toxicology and Carcinogenesis Studies of 1,2,3-Trichloropropane (CAS No. 96-18-4) in F344 Rats and $B6C3F_1$ Mice (Gavage Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-385 Toxicology and Carcinogenesis Studies of Methyl Bromide (CAS: 74-83-9) in $B6C3F_1$ Mice (Inhalation Studies)

Methyl bromide is widely used as a fumigant and pesticide. Toxicology and carcinogenesis studies were conducted by exposing groups of male and female $B6C3F_1$ mice to methyl bromide (99.8% pure) by inhalation 6 hours per day, 5 days per week, for 14 days, 6 weeks, 13 weeks, or 2 years. Six-week and 13-week inhalation toxicity studies in F344/N rats were conducted concurrently with the mouse studies. Hematology parameters were measured during the 6-week, 13-week, and 2-year studies. Quantitative neurobehavioral testing was performed during the 14-day, 13-week and 2-year studies. Genetic toxicology studies were conducted for gene mutation induction in *Salmonella typhimurium* and for induction of sister chromatid exchanges in mouse bone marrow cells and of micronuclei from peripheral blood erythrocytes.

14-Day Studies: Groups of five $B6C3F_1$ mice of each sex were exposed to 0, 12, 25, 50, 100, or 200 ppm methyl bromide by inhalation 6 hours per day, 5 days per week for 2 weeks. Only four female mice and one male mouse survived 10 exposures at 200 ppm. No deaths occurred at the lower doses. Neurobehavioral effects including trembling and paralysis were noted in all groups, but were most pronounced in the three highest dose groups. Red urine was noted in the mice exposed to 200 ppm.

13-Week Studies: Groups of 10 mice of each sex were exposed to 0, 10, 20, 40, 80, or 120 ppm methyl bromide by inhalation 6 hours per day, 5 days per week for 13 weeks. Additional groups of eight to 17 mice were concurrently exposed for neurobehavioral and genetic toxicology studies. The final mean body weight of males exposed to 120 ppm was significantly (12%) lower than that of the controls. Four of 24 males exposed to 120 ppm died during the study.

Groups of 10 rats of each sex were exposed to 0, 30, 60, or 120 ppm methyl bromide by inhalation 6 hours per day, 5 days per week for 13 weeks. Additional groups of eight rats were concurrently exposed for neurobehavioral studies. Final mean body weights of rats exposed to 120 ppm were 12% lower than those of the controls for males and 13% lower for females. No rats died as a result of methyl bromide exposure during the studies.

Special 6-Week Target Organ Toxicity Studies: Neither the 14-day nor the 13-week studies provided strong evidence for specific organ toxicity. Six-week studies were therefore conducted to identify target organs for the 2-year studies. Groups of 20 rats and mice of each sex were exposed to methyl bromide by inhalation for 6 hours per day, 5 days per week for 6 weeks at a dose of 160 ppm. Mortality rates exceeded 50% in the male mice after eight exposures, in female mice after six exposures, and in male rats after 14 exposures. Only the female rat group survived 30 exposures with less than 50% mortality. The study identified the brain, kidney, nasal cavity, heart, adrenal gland, liver, and testis as the primary organs to examine for toxicity in the 2-year methyl bromide inhalation studies.

2-Year Studies: Groups of 70 $B6C3F_1$ mice of each sex were exposed to methyl bromide by inhalation at 0, 10, 33, or 100 ppm for 6 hours per day, 5 days per week for up to 103 weeks. Additional groups of 16 mice were included for neurobehavioral evaluations throughout the 2-year studies. By 20 weeks (139 days), 27 males and 7 females exposed to 100 ppm had died and methyl bromide exposure was discontinued for the remaining mice in this dose group. Ten female mice from the 100 ppm group predesignated for the 15-month interim evaluation were killed on schedule and all other high-dose animals were allowed to live to term (24 months) for evaluation of chronic toxicity and carcinogenicity. Clinical signs indicative of neurotoxicity, including tremors, abnormal posture, tachypnea, and hind leg paralysis, persisted in these high-dose mice until the end of the studies.

Final mean body weights of surviving 100 ppm males and females were markedly lower (33% and 31%) than those of the controls. Neurobehavioral changes occurred in male and female mice initially exposed to 100 ppm methyl bromide, with more pronounced changes observed in males. In general, these animals were less active and manifested a heightened sensitivity in the startle response than mice in other dose groups.

Exposure to methyl bromide was not carcinogenic under the conditions of these studies. However, there was an increase in the incidence of several nonneoplastic lesions in the brain, heart, bone (sternum), and nose. Degenerative changes in the cerebellum and cerebrum occurred in males and females exposed to 100 ppm. Myocardial degeneration and cardiomyopathy were observed in the hearts of mice exposed to 100 ppm. An increased incidence of sternal dysplasia was seen in treated animals, particularly in those exposed to 100 ppm. An increased incidence of olfactory epithelial necrosis and metaplasia within the nasal cavity was seen in the mice exposed to 100 ppm, particularly males.

Genetic Toxicology: Methyl bromide was positive for induction of gene mutations in *Salmonella typhimurium* strain TA100, with and without exogenous metabolic activation; negative results were obtained with TA98 in this assay. *In vivo*, methyl bromide induced sister chromatid exchanges in bone marrow cells and micronuclei in peripheral erythrocytes of female mice exposed by inhalation for 14 days. No significant increase in either sister chromatid exchanges or micronuclei was observed in male or female mice exposed to methyl bromide by inhalation for 4, 8, or 12 weeks.

Conclusions: Under the conditions of these 2-year inhalation studies, methyl bromide caused degenerative changes in the cerebellum and cerebrum, myocardial degeneration and cardiomyopathy, sternal dysplasia, and olfactory epithelial necrosis and metaplasia. Toxic effects persisted although exposure to methyl bromide in the 100 ppm group terminated after 20 weeks. There was *no evidence of carcinogenic activity* of methyl bromide in male or female $B6C3F_1$ mice exposed to 10, 33, or 100 ppm.

Synonym: Bromomethane

Report Date: March 1992

TR-386 Toxicology and Carcinogenesis Studies of Tetranitromethane (CAS No. 509-14-8) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

Tetranitromethane is a volatile contaminant formed during the manufacture of TNT and has been used as a rocket fuel and biochemical reagent. Toxicology and carcinogenesis studies were conducted in F344/N rats and $B6C3F_1$ mice of each sex by whole body exposure to tetranitromethane vapor (greater than 99% pure), 6 hours per day, 5 days per week for 14 days, 13 weeks, or 2 years. Additional groups of male mice were exposed to tetranitromethane for evaluation at 1 year. Genetic toxicology studies were performed in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: Exposure concentrations ranged from 2 to 25 ppm for rats and from 2 to 50 ppm for mice. All rats exposed to 25 ppm and all mice exposed at the top concentration of 50 ppm died by day 2; reduced survival was seen in mice exposed to 25 ppm and in rats exposed to 10 ppm. Pulmonary edema in rats and inflammation of the lung in mice were seen in those animals in the 25- and 50-ppm exposure groups examined microscopically.

Thirteen-Week Studies: Exposure concentrations ranged from 0.2 to 10 ppm for rats and mice. No exposure-related deaths occurred in rats. The final mean body weight of rats exposed to 10 ppm was 16% lower than that of controls for males and 6% lower for females. Exposure-related histologic effects included squamous metaplasia of the respiratory epithelium of the nasal mucosa and chronic inflammation of the lung.

No deaths of mice could be clearly related to exposure to tetranitromethane. The final mean body weights of mice exposed to 5 or 10 ppm were 5% or 12% lower than that of controls for males and 9% or 12% lower for females. Exposure-related histologic effects in mice included inflammation and squamous metaplasia of the respiratory epithelium of the nasal mucosa and hyperplasia of the bronchiolar epithelium.

Based on the incidences and severity of lesions in the respiratory at the higher concentrations used in the 13-week studies, exposure concentrations chosen for the 2-year studies were 0, 2, and 5 ppm for groups of 50 rats of each sex and 0, 0.5, and 2 ppm for groups of 50 mice of each sex. Additional groups of 6 or 10 male mice were exposed at concentrations of 0, 0.5, or 2 ppm for 1 year.

Body Weights and Survival in the Two-Year Studies: Mean body weights of male and female rats exposed to 5 ppm were approximately 5%-15% lower than those of controls after week 70. Survival of rats at 104 weeks was as follows: male: control, 18/50; 2 ppm, 17/50; 5 ppm, 4/50; female: 25/50; 34/50; 15/50; survival of rats at the top concentration was reduced due to neoplasia.

Mean body weights of exposed mice were variable and ranged as much as 10% below those of controls during the second year of the studies. Survival of exposed male mice at 104 weeks was significantly lower than that of controls due to neoplasia (control, 37/50; 0.5 ppm, 26/50; 2 ppm, 15/50). Survival of female mice was not significantly affected by exposure to tetranitromethane (31/50; 28/50; 24/50).

Neoplastic and Nonneoplastic Effects in the Two-Year Studies: Effects of exposure to tetranitromethane were limited to the respiratory tract. Hyperplasia of the alveolar and bronchiolar epithelium was observed at increased incidences in exposed rats. The incidence of alveolar/bronchiolar adenomas and carcinomas were markedly increased in exposed male and female rats, with carcinomas (many of which metastasized to other sites) occurring in nearly all rats exposed to the top concentration of 5 ppm (adenomas or carcinomas—male: control,

1/50; 2 ppm, 33/50; 5 ppm, 46/50; female: 0/50; 22/50; 50/50). Many of the rats exposed to 5 ppm also had squamous cell carcinomas of the lung (male: 0/50; 1/50; 19/50; female: 0/50; 1/50; 12/50).

Hyperplasia of the respiratory epithelium and chronic inflammation of the nasal mucosa were observed at increased incidences in exposed male and female rats. Squamous metaplasia of the respiratory epithelium was increased in exposed male rats. No neoplasms of the nasal passage were seen.

In exposed mice, hyperplasia of the alveolar and bronchiolar epithelium was observed at increased incidences. Alveolar/bronchiolar neoplasms, primarily carcinomas (many of which metastasized to other sites), were increased in exposed male and female mice (male: control, 12/50; 0.5 ppm, 27/50; 2 ppm, 47/50; female: 4/49; 24/50; 49/50).

Chronic inflammation of the nasal mucosa and hyperplasia and squamous metaplasia of the respiratory epithelium of the nasal cavity occurred at increased incidences in female mice exposed to 2 ppm. No primary neoplasms of the nasal passage were observed in mice.

Oncogene Analysis: DNA from 14/19 rat and 4/4 mouse lung neoplasms caused morphologic transformation after transfection into cultured NIH/3T3 fibroblasts. The transforming gene from both rat and mouse lung neoplasms was determined by Southern blot analysis to be an activated K-*ras* oncogene.

Genetic Toxicology: Tetranitromethane was mutagenic in *S. typhimurium* strains TA98, TA100, and TA1535 with and without exogenous metabolic activation (S9); no mutagenic activity was observed in TA1537 with or without S9. Chromosomal aberrations were observed in CHO cells treated in vitro with tetranitromethane in the presence of S9. Sister chromatid exchanges were induced in CHO cells in the absence of S9.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity* of tetranitromethane for male and female F344/N rats and male and female B6C3F$_1$ mice, based on increased incidences of alveolar/bronchiolar neoplasms in both species and squamous cell carcinomas of the lung in rats.

Chronic inflammation of the nasal mucosa was related to exposure in rats and female mice, and hyperplasia and squamous metaplasia of the respiratory epithelium were increased in exposed male rate.

Synonym: TNM

Report Date: March 1990

TR-387 Toxicology and Carcinogenesis Studies of *dl*-Amphetamine Sulfate (CAS No. 60-13-9) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

dl-Amphetamine sulfate is used for the treatment of narcolepsy in adults and behavioral syndromes in children. Toxicology and carcinogenesis studies were conducted by administering *dl*-amphetamine sulfate (USP grade) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

14-Day Studies: The chemical was administered at dietary concentrations of 0, 47, 94, 188, 375, or 750 ppm for rats and 0, 125, 250, 500, 1,000, or 2,000 ppm for mice. Decreased body weight gain was seen at the higher concentrations, but no chemical-related deaths or toxic lesions were observed.

13-Week Studies: The chemical was administered at dietary concentrations of 0, 47, 94, 188, 375, or 750 ppm for rats and 0, 125, 250, 500, 1,000, or 2,000 ppm for mice. None of the rats died, but 6/10 male mice and 7/10 female mice that received 2,000 ppm, 3/10 male mice that received 1,000 ppm, and 8/10 male mice that received 500 ppm died before the end of the studies. Decreased body weight gain and hyperactivity were seen in dosed rats and mice. Final body weights of rats receiving 188 ppm or more were 62% to 89% those of controls, and final body weights of mice receiving 250 ppm or more were 70% to 86% those of controls. There were no lesions that were considered to be a primary effect of the chemical.

Based on decreased body weight gain and hyperactivity in the 13-week studies, 2-year studies were conducted by feeding diets containing 0, 20 or 100 ppm *dl*-amphetamine sulfate to groups of 50 rats or 50 mice of each sex.

Body Weights and Survival in the 2-Year Studies: No significant differences in survival were observed between any groups of rats or mice (male rats: control, 30/50; low dose, 31/50; high dose, 33/50; female rats: 33/50; 42/50; 37/50; male mice: 48/50; 48/50; 49/50; female mice: 35/50; 36/50; 44/50).

Final body weights of dosed rats and mice were decreased relative to those of controls. Final body weights were 92% and 86% those of controls for low- and high-dose male rats, 89% and 70% those of controls for low- and high-dose female rats, 85% and 72% those of controls for low- and high-dose male mice, and 81% and 66% those of controls for low- and high-dose female mice. Hyperactivity was observed in all dosed groups.

Feed consumption was similar among control and exposed groups with the exception of high-dose female rats (84% of controls) and high-dose male mice, for which hyperactivity resulted in scattering of feed and overestimation of feed consumption. The average amount of *dl*-amphetamine sulfate consumed per day was estimated to be 1 or 5 mg/kg for low- and high-dose rats, 4 or 30 mg/kg for low- or high-dose male mice, and 3 or 19 mg/kg for low- or high-dose female mice.

Nonneoplastic and Neoplastic Effects in the 2-Year Studies: Myelofibrosis, cataracts, and retinal atrophy in female rats, and ovarian atrophy in female mice occurred in a larger proportion of high-dose animals than in controls.

Dose-related increases in neoplasms did not occur in rats or mice receiving amphetamine. The administration of *dl*-amphetamine sulfate was associated with

decreases in the incidence of total neoplasms and in the incidences of certain site-specific neoplasms, including pheochromocytomas of the adrenal gland in male rats (23/49, 15/44, 7/50), fibroadenomas of the mammary gland in female rats (21/50, 11/50, 2/50), adenomas of the anterior pituitary gland in male and female rats and female mice (male rats: 15/49, 15/48, 9/49; female rats: 31/50, 24/48, 19/50; female mice: 12/49, 6/49, 1/46), endometrial stromal polyps of the uterus of female rats (10/50, 6/50, 3/50), adenomas or carcinomas (combined) of the liver in male and female mice (male: 14/50, 12/50, 2/50; female: 5/50, 1/50, 1/47), adenomas of the harderian gland in male and female mice (male: 4/50, 2/50, 0/50; female: 5/50, 2/50, 0/47), and adenomas or carcinomas (combined) of the lung in male and female mice (male: 8/50, 3/50, 4/50; female: 8/50, 6/50, 1/47).

Genetic Toxicology: *dl*-Amphetamine sulfate was tested for induction of gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without exogenous metabolic activation (S9); the only response observed was in strain TA98 in the presence of S9, and it was judged to be equivocal. No induction of sister chromatid exchanges or chromosomal aberrations occurred in Chinese hamster ovary cells treated with amphetamine sulfate in either the presence or the absence of S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* of *dl*-amphetamine sulfate for male or female F344/N rats or male or female B6C3F$_1$ mice fed 20 or 100 ppm. The administration of *dl*-amphetamine sulfate was associated with decreased body weight. There were decreased incidences of total neoplasms in dosed rats and mice, of adrenal pheochromocytomas in male rats, of mammary gland fibroadenomas and uterine polyps in female rats, of pituitary gland adenomas in male and female rats and female mice, and of harderian gland adenomas, liver neoplasms, and lung neoplasms in male and female mice.

Synonyms: (±)-amphetamine sulfate; (±)-2-amino-1-phenylpropane sulfate; amphetamine sulfate; deoxynorephedrine; desoxynorephedrine; (±)-α-methylphenethylamine sulfate; (±)-phenisopropylamine sulfate; β-phenyl isopropylamine

Trade Names: Acedron; Adipan; Adiparthrol; Aketdrin; Aktedrin; Alentol; Amfetamina; Amphaetamin; Amphamed; Amphatamin; Amphate; Amphedrine; Amphetaminum; Amphezamin; Amphoids-S; Anara; Anfetamina; Anorexine; Astedin; Benzafinyl; Benzamphetamine; Benzebar; Benzedrina; Benzedryna; Benzolone; Benzpropamine; Betafen; Betaphen; Bluzedrin; Centramina; Didrex; Dietamine; Durophet; Elastonin; Elastonon; Euphobine; Euphodine; Euphodyn; Fabedrine; Fenamin; Fenara; Fenedrin; Fenopromin; Halloo-Wach; Ibiozedrine; Isamin; Isoamin; Isoamyne; Isomyn; Leodrin; Levonor; Linampheta; Mecodrin; Mimetina; Monetamine; Noclon; Norephedrane; Norphedrane; Novydrine; Oktedrin; Oraldrina; Ortenal; Orthedrin; Percomon; Pharmamedrine; Pharmedrine; Phenamine; Phenedrine; Phenopromin; Phenpromin; Profamina; Profetamine; Propenyl; Propisamine; Psychedrine; Psychedryna; Psychedrinum; Psychoton; Racephen; Rhinalator; Sedolin; Simpamina; Simpamine; Simpatedrin; Stimulan; Sympametin; Sympamine; Sympatedrine; Synsatedrine; Theptine; Vapedrine; Weckamine; Zedrine

Slang for Amphetamines: bennies; benzies; cartwheels; hearts; peaches; roses

TR-388 Toxicology and Carcinogenesis Studies of Ethylene Thiourea (CAS: 96-45-7) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

Ethylene thiourea is a white crystalline solid used extensively in the rubber industry as an accelerator in the vulcanization of elastomers. It is also a trace contaminant and metabolic degradation product of a widely used class of ethylene bisdithiocarbamate fungicides. Ethylene thiourea is known to produce thyroid neoplasms in rats and liver neoplasms in mice following long-term administration; thus, it was chosen by the National Toxicology Program in an investigation of the potential value of perinatal exposures in assessing chemical carcinogenicity.

Chronic toxicity and carcinogenicity studies of ethylene thiourea, 99% pure, were conducted in F344/N rats and B6C3F$_1$ mice of each sex. The studies were designed to determine 1) the effects of ethylene thiourea in rats and mice receiving adult exposure only (a typical carcinogenicity study), 2) the toxic and carcinogenic effects of ethylene thiourea on rats and mice receiving perinatal exposure only (dietary exposure of dams prior to breeding and throughout gestation and lactation), and 3) the effects of combined perinatal and adult exposure to ethylene thiourea.

Studies in F344/N Rats: In a preliminary study to determine the perinatal dietary concentrations for the 2-year studies, female F344/N rats were fed 0, 8, 25, 83, or 250 ppm ethylene thiourea in the feed beginning 2 weeks prior to breeding and continuing throughout gestation and lactation, and the pups were fed at these same concentrations up to 9 weeks postweaning. Based on decreased survival of rat pups between postnatal days 0 to 4 and reduction in body weight gains in male weanling rats receiving 250 ppm, dietary concentrations of 0, 9, 30, and 90 ppm were selected for the perinatal (F_0) exposure levels in the 2-year studies. Groups of 10 male and 10 female rats, 8 to 9 weeks of age, were fed diets containing 0, 60, 125, 250, 500, or 750 ppm ethylene thiourea for 13 weeks to determine the adult dietary concentrations. Because of reduced weight gains and decreased feed consumption in rats receiving 500 or 750 ppm ethylene thiourea, dietary concentrations of 0, 25, 83, and 250 ppm were selected for the adult (F_1) exposure during the 2-year studies.

In the 2-year studies, perinatal and adult exposures to ethylene thiourea were applied separately and together to groups of male or female rats as shown in the following table.

The principal toxic effects of ethylene thiourea involved the thyroid gland. Serum levels of thyroxine (T_4) and/or triiodothyronine (T_3) were significantly decreased in rats receiving adult concentrations of 83 or 250 ppm, and thyrotropin (thyroid-stimulating hormone, TSH) was significantly increased at these concentrations. In male and female rats receiving adult-only exposure of 83 or 250 ppm, the incidences of follicular cell hyperplasia or follicular cell adenoma of the thyroid gland were significantly increased relative to the controls. The incidences of follicular cell carcinoma were significantly increased in the 250 ppm groups, and carcinomas occurred more frequently in males than in females.

Perinatal-only exposure to 90 ppm had no effect on the incidence of thyroid neoplasms in these studies, although there was a marginal increase in follicular cell hyperplasia relative to the controls. However, for groups of rats receiving combined perinatal and adult exposure (F_0:F_1), males and females receiving concentrations of 90:250 ppm ethylene thiourea had significantly increased incidences of thyroid follicular cell neoplasms relative to those receiving adult-only exposure to 250 ppm. Further, groups of male rats receiving 90:83 ppm showed a significantly increased incidence of follicular cell hyperplasia. Final mean body weights of males and survival of males and females receiving combined perinatal (90 ppm) and adult (250 ppm) exposure were lower than those receiving adult-only exposure of 250 ppm.

Thus, in rats, combined perinatal and adult exposure slightly enhanced the toxicity and proliferative effects on the thyroid gland observed with adult-only exposure to ethylene thiourea.

Neoplasms of the Zymbal's gland were marginally increased in rats receiving 90:250 ppm (males - 0:0, 1/50; 90:250, 5/50; females - 0:0, 1/50; 90:250, 4/50). Mononuclear cell leukemia occurred with a significant trend in groups of male and female rats receiving perinatal exposure of 90 ppm and increasing adult concentrations (90:0, 90:83, and 90:250 ppm), and for female rats without perinatal exposure (0:0, 0:83, and 0:250 ppm). The incidences of mononuclear cell leukemia in males receiving 90:83 ppm and males and females receiving 90:250 ppm were statistically significant relative to the respective 0:0 ppm groups. Low incidences of renal tubule cell adenomas occurred in most dose groups of male rats, but not in the highest dose group or the controls.

Studies in B6C3F_1 Mice: In a preliminary study to determine the perinatal dietary concentrations for the 2-year studies, adult female C57BL/6N mice were fed 0, 33, 100, 330, or 1,000 ppm ethylene thiourea in the feed beginning 2 weeks prior to breeding and continuing throughout gestation and lactation and up to 9 weeks postweaning. Because of reduced survival of mouse pups at postnatal day 28 and lower final mean body weights in weanlings receiving perinatal exposure of 1,000 ppm, dietary concentrations of 0, 33, 110, and 330 ppm were selected for the perinatal exposure levels in the 2-year studies. Groups of 10 male and 10 female mice, 8 to 9 weeks of age, were fed diets containing 0, 125, 250, 500, 1,000, or 2,000 ppm ethylene thiourea for 13 weeks to determine the adult dietary concentrations. Moderately severe diffuse follicular cell hyperplasia in the thyroid gland and centrilobular cytomegaly of the liver occurred in mice receiving 2,000 ppm. Because the severity of the thyroid lesion (and degree of hypothyroidism) at this concentration was considered potentially life threatening in 2-year studies, dietary concentrations of 0, 100, 330, and 1,000 ppm ethylene thiourea were selected for adult exposure during the 2-year studies.

In the 2-year studies, perinatal and adult exposures to ethylene thiourea were applied separately and together to groups of male or female mice as shown in the following table.

The principal toxic effects of ethylene thiourea in mice occurred in the thyroid gland, liver, and pituitary gland. Serum levels of T_3 were significantly decreased in groups of mice receiving adult concentrations of 1,000 ppm; TSH was significantly increased in mice receiving 330 and 1,000 ppm. The incidences of follicular cell hyperplasia and neoplasia increased principally in males receiving 1,000 ppm and in females receiving 330 or 1,000 ppm. Follicular cell carcinomas were significantly increased in mice receiving 1,000 ppm. Incidences of centrilobular hepatocellular cytomegaly (males and females), hepatocellular adenoma (females), hepatocellular carcinoma (males and females), and adenoma or carcinoma combined (males and females) also were significantly increased in mice receiving F_1 concentrations of 330 or 1,000 ppm. In the pituitary gland, incidences of focal hyperplasia (males) or adenoma (males and females) of the pars distalis were significantly increased in groups of mice receiving 1,000 ppm ethylene thiourea.

Perinatal exposure to concentrations of 330 ppm had no effect on the incidences of nonneoplastic lesions or neoplasms in mice. For groups of mice receiving combined perinatal and adult exposure, females receiving F_0:F_1 concentrations of 330:330 ppm had significantly increased incidences of follicular cell adenoma relative to those receiving adult-only exposure to 330 ppm. Similarly, male mice receiving F_0:F_1 concentrations of 330:330 ppm had significantly increased incidences of follicular cell hyperplasia. Thus, in mice, perinatal exposure slightly enhanced the proliferative effects on the thyroid gland of adult exposure. There were no effects of perinatal exposure in mice at sites other than in the thyroid gland.

Conclusions: 2-Year Adult-Only Exposure:Under the conditions of these 2-year adult-only dietary exposures, there was *clear evidence of carcinogenic activity* of ethylene thiourea in male and female F344/N rats, as shown by increased incidences of thyroid follicular cell neoplasms. There was *clear evidence of carcinogenic activity* of ethylene thiourea in male and female B6C3F_1 mice as shown by increased incidences of thyroid follicular cell neoplasms, hepatocellular neoplasms, and adenomas of the pars distalis of the pituitary gland.

Nonneoplastic lesions associated with the administration of ethylene thiourea included follicular cell hyperplasia in rats and mice and follicular cell cytoplasmic vacuolation, centrilobular hepatocellular cytomegaly, and focal hyperplasia of the pars distalis of the pituitary gland in mice. Other effects associated with the administration of ethylene thiourea included decreased serum levels of T_4 and/or T_3 in rats and increased serum levels of TSH in rats and mice.

Perinatal-Only Exposure:Perinatal exposure alone had no effect on the incidences of neoplasms in rats or mice after 2 years. Animals may have been able to tolerate higher perinatal exposure concentrations.

Combined Perinatal and 2-Year Adult Exposures: Combined perinatal and 2-year adult dietary exposure to ethylene thiourea confirmed the findings of the 2-year adult-only exposures for the incidences of neoplasms in the thyroid gland of rats and mice and the liver and pituitary gland of mice. In male and female rats, combined perinatal and adult exposure to 90:250 ppm was associated with marginal increases, relative to the untreated (0:0 ppm) controls, in Zymbal's gland neoplasms and mononuclear cell leukemia, which may have been related to chemical administration. In rats receiving adult exposure to 250 ppm ethylene thiourea, perinatal exposure to 90 ppm was associated with a slightly enhanced incidence of thyroid neoplasms compared to adult-only exposure. However, increasing perinatal exposure from 0 to 90 ppm had no effect on incidences of thyroid neoplasms in rats receiving adult exposure to 83 ppm. Increasing perinatal exposure from 0 to 330 ppm was associated with a marginally increased incidence of thyroid neoplasms in female mice receiving adult exposure to 330 ppm, but there were no enhancing effects of perinatal exposure in mice receiving adult exposure to 1,000 ppm.

Synonyms: 2-Imidazolidinethione; Imidazoline-2-thiol; 2-mercaptoimidazoline; *N,N'*-ethylenethiourea; 1,3-ethylenethiourea; 2-imadazoline-2-thiol

Report Date: March 1992

TR-389 Toxicology and Carcinogeneis Studies of Sodium Azide (CAS: 26628-22-8) in F344 Rats (Gavage Studies)

Sodium azide is a white crystalline solid used in the manufacture of the explosive lead azide. It is the principal chemical used to generate nitrogen gas in automobile safety airbags and airplane escape chutes and is a broad-spectrum biocide used in both research and agriculture.

Toxicology and carcinogenicity studies were conducted by administering sodium azide (greater than 99% pure) in distilled water by gavage to groups of male and female F344/N rats once daily, 5 days per week for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary cells.

14-Day Studies: Rats received 0, 5, 10, 20, 40, or 80 mg/kg sodium azide. All male and female rats receiving 40 or 80 mg/kg and two of five female rats receiving 20 mg/kg died during the first week of the studies. Clinical findings of toxicity included lethargy and inactivity. No grossly observable lesions were present in any of the dose groups.

13-Week Studies: Rats received 0, 1.25, 2.5, 5, 10, or 20 mg/kg sodium azide. Seven of 9 males and all 10 females receiving 20 mg/kg died before the end of the studies. Final mean body weights of treated rats were within 10% of those of the controls. Compound-related clinical findings of toxicity in the 20 mg/kg dose groups included lethargy and labored breathing. Histopathologic lesions induced by sodium azide were limited to the brain (necrosis of the cerebrum and thalamus) and lung (congestion, hemorrhage, and edema), and were observed in rats receiving 20 mg/kg that died during the studies.

Body Weights, Feed Consumption, and Survival in the 2-Year Studies: Because compound-related deaths were observed in the groups receiving 20 mg/kg in the 13-week studies, lower dose levels were used in the 2-year studies. Two-year studies were conducted by administering 0, 5, or 10 mg/kg sodium azide to groups of 60 male and 60 female rats. Dose-related depression in mean body weight was observed throughout the study period. Mean feed consumption values in low- and high-dose groups were lower than control values. Survival of high-dose rats of each sex was significantly ($P<0.05$) lower than controls (males-control, 24/60; low-dose, 27/60; high-dose, 9/60; females-37/60; 43/60; 21/59). The reduced survival was attributed to brain necrosis and cardiovascular collapse induced by sodium azide.

Neoplastic and Nonneoplastic Effects in the 2-Year Studies: There were no compound-related increases in incidences of neoplasms in rats. Significantly decreased incidences were observed for certain neoplasms, including mononuclear cell leukemia in male rats (control, 33/60; low-dose, 28/60; high-dose, 14/60), adrenal gland pheochromocytoma in male rats (26/55; 16/56; 6/54), mammary gland fibroadenoma in female rats (20/60; 11/60; 8/59), and pituitary gland neoplasms in female rats (37/60; 28/60; 17/59). These decreases reflected to some extent, but could not be attributed solely to, the reduced survival of the high-dose groups. Compound-related nonneoplastic brain lesions (necrosis of the cerebrum and thalamus) were observed at significantly ($P<0.001$) increased incidences in high-dose male and female rats. The increased incidence of lung congestion observed in this dose group was considered due to cardiovascular collapse secondary to brain necrosis.

Genetic Toxicology: Sodium azide was mutagenic in *Salmonella typhimurium* strains TA100 and TA1535, with or without exogenous metabolic activation (S9); it was not mutagenic in strain TA1537 or TA98. In cytogenetic tests with Chinese hamster ovary cells, sodium azide induced sister chromatid exchanges, but not chromosomal aberrations, in the presence and the absence of S9.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of sodium azide in male or female F344/N rats administered 5 or 10 mg/kg.

Sodium azide induced necrosis in the cerebrum and the thalamus of the brain in both male and female rats.

Synonyms: Azide, Azium, Smite

Report Date: September 1991

TR-390 Toxicology and Carcinogenesis Studies of 3,3'-Dimethylbenzidine Dihydrochloride (CAS No. 612-82-8) in F344/N Rats (Drinking Water Studies)

3,3'-Dimethylbenzidine dihydrochloride is one of five chemicals being evaluated in 2-year carcinogenicity and toxicity studies as part of the NTP's Benzidine Dye Initiative. This Initiative was designed to evaluate representative benzidine congeners, benzidine congener-derived dyes, and benzidine-derived dyes. 3,3'-Dimethylbenzidine dihydrochloride was nominated for study because of the potential for human exposure during production of bisazobiphenyl dyes and because benzidine, a structurally related chemical, is a known human carcinogen.

Toxicology and carcinogenesis studies were conducted by administering 3,3'-dimethylbenzidine dihydrochloride (approximately 99% pure) in drinking water to groups of F344/N rats of each sex for 14 days, 13 weeks, or 9 or 14 months. The 14-month exposures were planned as 24-month exposures but were terminated early because of rapidly declining animal survival, due primarily to neoplasia. These studies were performed only in rats because similar studies were being performed in mice at the National Center for Toxicological Research (NCTR). Hematologic and serum chemical analyses and thyroid hormone determinations were conducted in conjunction with the 13-week and 9-month studies. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary (CHO) cells, and *Drosophila melanogaster*.

14-Day Studies: Rats were exposed to 3,3'-dimethylbenzidine dihydrochloride in drinking water at doses ranging from 600 to 7,500 ppm. All five males and one female in the 7,500 ppm group and 1/5 males in the 5,000 ppm group died. Final mean body weights were decreased in males receiving 1,250 ppm or more and in all exposed females, and final mean body weights of animals receiving 2,500 ppm or more were lower than initial weights. Water consumption decreased with increasing chemical concentration. Compound-related effects observed in rats receiving 5,000 ppm or more included minimal to slight hepatocellular necrosis, accumulation of brown pigment (presumably bile) in individual hepatocytes, increased severity of nephropathy relative to controls, and severe lymphocytic atrophy of the thymus. Treated animals also showed an increased severity of atrophy of the bone marrow relative to controls, varying degrees of lymphocytic atrophy of the mandibular and mesenteric lymph nodes and spleen, increased vacuolization and necrosis of cells of the adrenal cortex, focal acinar cell degeneration in the pancreas, and, in males, increased immature sperm forms in the testis and epididymis.

13-Week Studies: 3,3'-Dimethylbenzidine dihydrochloride was administered in drinking water at doses of 300, 500, 1,000, 2,000, and 4,000 ppm. All rats receiving 4,000 ppm and 4/10 males and 1/10 females receiving 2,000 ppm died before the end of the studies. Depressions in final mean body weight relative to controls ranged from 12% to 48% for males and from 9% to 42% for females. Water consumption decreased with increasing dose. At compound concentrations of 300 to 2,000 ppm, mean water consumption was 29% to 83% of control values. Compound-related effects included an increase in the severity of nephropathy relative to controls; hepatocellular necrosis and accumulation of brown pigment (presumably bile) in sinusoidal lining cells; lymphocytic atrophy of the thymus, spleen, and mandibular and mesenteric lymph nodes; atrophy of the bone marrow in the higher-dose groups; degeneration of pancreatic acinar cells; and, in males, immature sperm forms in the testis and epididymis.

Decreases in serum triiodothyronine (T_3) values were observed in exposed females, and decreases in mean thyroxin (T_4) concentrations in exposed males and females; no significant changes were observed in thyroid stimulating hormone (TSH) levels in exposed rats.

Based on the decreased survival, reductions in water consumption and body weight gain, and chemical-induced hepatocellular and renal lesions observed in the 13-week studies, the doses selected for the 9- and 14-month drinking water studies of 3,3'-dimethylbenzidine dihydrochloride were 0, 30, 70, and 150 ppm. Seventy rats of each sex were used in the control group, 45 in the low-dose group, 75 in the mid-dose group, and 70 in the high-dose group.

9-Month Studies: Ten rats of each sex in the control and 150 ppm dose groups were evaluated after 9 months. Chemical-related effects observed in exposed animals included alveolar/bronchiolar carcinoma in one male, basal cell carcinoma of the skin in one male, a squamous cell carcinoma of the oral cavity in one female, preputial gland carcinoma in two males, clitoral gland carcinoma in three females, adenocarcinoma of the small intestine in two males, Zymbal's gland carcinoma in two males and three females, hepatocellular carcinoma in two males, and adenomatous polyps of the large intestine in three males. Other effects seen in dosed rats included focal cellular alteration in the liver, lymphoid atrophy in the spleen, and increased severity of nephropathy relative to controls. An increase in serum T3 values was observed in exposed males, and a decrease in mean T4 concentrations in exposed males and females. TSH concentrations were increased in exposed male and female rats.

Body Weights and Survival in the 14-Month Studies: The average amount of 3,3'-dimethylbenzidine

dihydrochloride consumed per day was approximately 1.8, 4.0, or 11.2, mg/kg for low-, mid-, or high-dose male rats and 3.0, 6.9, or 12.9 mg/kg for low-, mid-, or high-dose female rats. The mean body weight of high-dose males was about 85% of the control value by week 28. By the end of the study, mean body weights of low-, mid-, and high-dose males were 97%, 92%, and 70% of the control values, respectively. Mean body weights of high- and mid-dose females were about 85% of the control values at week 32 and week 44, respectively. At the end of the study, mean body weights of exposed females were about 94%, 81%, and 74% of the control values for low-, mid-, and high-dose groups, respectively. Because of extensive neoplasia, many exposed males and females were dying or were sacrificed moribund in the first year, and all high-dose males died by week 55. The studies were terminated at weeks 60 to 61, at which time the group survivals were male: control, 60/60, low dose, 41/45; mid dose, 50/75; high dose, 0/60; female: 59/60; 39/45; 32/75; 10/60.

Nonneoplastic Effects in the 14-Month Studies: Increases in nonneoplastic lesions in dosed rats included cystic degeneration and foci of cellular alteration in the liver; exacerbation of nephropathy; and focal or multifocal hyperplasia of the Zymbal's gland, preputial and clitoral glands, and alveolar epithelium.

Neoplastic Effects in the 14-Month Studies: Neoplasms were observed in exposed rats at many sites: skin, Zymbal's gland, preputial and clitoral glands, liver, oral cavity, small and large intestine, mammary gland, lung, brain, and mesothelium. The incidence of these neoplastic effects in male and female rats is summarized in the table at the end of this section (see page 8 of the Technical Report).

Genetic Toxicology: 3,3'-Dimethylbenzidine dihydrochloride was mutagenic in *Salmonella typhimurium* strain TA98 with exogenous metabolic activation; it was not mutagenic in strains TA100, TA1535, or TA97 with or without activation. 3,3'-Dimethylbenzidine dihydrochloride induced sister-chromatid exchanges (CHO) and chromosomal aberrations in CHO cells in the absence of exogenous metabolic activation; these effects were not evident in test with S9 activation. Sex-linked recessive lethal mutations were induced in germ cells of adult male *Drosophila melanogaster* administered 3,3'-dimethylbenzidine dihydrochloride in feed or by injection. No reciprocal translocations occurred in D. melanogaster germ cells following exposure to 3,3'-dimethylbenzidine dihydrochloride.

Conclusions: Under the conditions of these 14-month drinking water studies, there was *clear evidence of carcinogenic activity* of 3,3'-dimethylbenzidine dihydrochloride for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity, small and large intestine, lung, and mesothelium. Increased incidences of neoplasms of the brain may have been related to chemical administration. There was *clear evidence of carcinogenic activity* for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity, small and large intestine, mammary gland, and lung. Increased incidences of neoplasms of the brain and mononuclear cell leukemia may have been related to chemical administration.

Synonyms: o-tolidine dihydrochloride; 3,3'-dimethylbiphenyl-4,4'-diamine dihydrochloride; 3,3'-dimethylbiphenyl-4,4'-biphenyldiamine dihydrochloride; 4,4'-diamino-3,3'-dimethylbiphenyl dihydrochloride

Report Date: June 1991

TR-391 Toxicology and Carcinogenesis Studies of Tris(2-chloroethyl) Phosphate (CAS No. 115-96-8) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Tris(2-chloroethyl) phosphate (TRCP), a flame-retardant plasticizer used in plastics, polymeric foams, and synthetic fibers, was studied as part of the National Toxicology Program's class study of trisalkyl phosphate flame retardants. Toxicology and carcinogenesis studies were conducted by administering TRCP (approximately 98% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days, 16 weeks, or 2 years. Genetic toxicology studies were performed in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

16-Day Studies: There were no chemical-related deaths, differences in final mean body weight, or histopathological lesions in rats receiving 22 to 350 mg/kg TRCP or in mice receiving 44 to 700 mg/kg TRCP for 12 doses over 16 days. Serum cholinesterase activity in female rats receiving 175 or 350 mg/kg TRCP was reduced slightly (80% of control levels), but enzyme activity in dosed male rats and in mice was similar to that in controls.

16-Week Studies: Rats received 22 to 350 mg/kg TRCP for 16 weeks (female) or 18 weeks (male). Several male and female rats in the 175 or 350 mg/kg dose groups died from chemical toxicity. Final mean body weights of female rats receiving 350 mg/kg were 20% greater than those of controls; final mean body weights of the remaining groups of dosed female rats and dosed male rats were similar. Chemical-related neuronal necrosis occurred in the hippocampus and thalamus of female rats and, to a lesser extent, of male rats. Serum cholinesterase activity was reduced in females receiving 175 or 350 mg/kg TRCP.

There were no chemical-related deaths, differences in final mean body weight, or differences in cholinesterase activity in mice receiving 44 to 700 mg/kg TRCP for 16 weeks. Tubule epithelial cells with enlarged nuclei (cytomegaly and karyomegaly) were observed in the kidneys of high-dose (700 mg/kg) male and female mice.

2-Year Studies: The 2-year studies in rats were conducted by administering 0, 44, or 88 mg/kg TRCP to groups of 60 males and females, 5 days per week for up to 104 weeks; 9 or 10 rats of each dose group were evaluated at 66 weeks. The survival of high-dose male and female

rats was reduced relative to that of controls. Final mean body weights of surviving rats were similar to those of controls. The principal chemical-related effects occurred in the kidney and brain of dosed rats. Focal hyperplasia of the renal tubule epithelium and renal tubule adenomas were markedly increased in male rats receiving 88 mg/kg TRCP and, to a lesser extent, in female rats (renal tubule hyperplasia, male rats: 0/50; 2/50; 24/50; female rats: 0/50; 3/50; 16/50; renal tubule adenoma, male rats: 1/50; 5/50; 24/50; female rats: 0/50; 2/50; 5/50). Renal tubule carcinomas occurred in one control and one high-dose male rat. Degenerative lesions consisting of gliosis, mineralization, hemorrhage, and/or hemosiderin accumulation occurred in the cerebrum and brain stem of more than 50% of female rats receiving 44 or 88 mg/kg TRCP; similar lesions were seen in only a few dosed males. Slightly increased incidences of thyroid gland follicular cell neoplasms (male rats: 5/50; 14/50; 13/50; female rats: 14/50; 16/50; 20/50) occurred in dosed males and females, but it is uncertain whether these were related to chemical administration.

The 2-year studies in mice were conducted by administering 0, 175, or 350 mg/kg TRCP to groups of 60 males and females, 5 days per week for up to 104 weeks; 8 to 10 mice of each sex per dose group were evaluated at 66 weeks. There were no significant differences in survival between dosed and control groups of either sex, and final mean body weights of mice were similar among all groups. The principal chemical-related effects occurred in the kidney, in which nuclear enlargement (karyomegaly) of tubule epithelial cells was present in approximately 80% of high-dose mice. In the original diagnosis, renal tubule adenomas were seen in one control male, one high-dose male, and one low-dose female. A carcinoma was also seen in one high-dose male. In a subsequent examination of step sections of all the mouse kidneys, adenomas were found in one low-dose male and two high-dose males. The incidences of renal tubule neoplasms in the original and step sections combined were 1/50, 1/50, and 4/50 for males. Female mice receiving TRCP demonstrated a marginally increased incidence of neoplasms (primarily adenomas) of the harderian gland (3/50; 8/50; 7/50); in addition, three harderian gland neoplasms occurred in high-dose female mice evaluated after 66 weeks.

Genetic Toxicology: TRCP was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 with or without exogenous metabolic activation (S9), and it tested negative for the induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells. TRCP produced an equivocal response in the presence of S9 for the induction of sister chromatid exchanges (SCE) in CHO cells.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* for male and female F344/N rats receiving tris(2-chloroethyl) phosphate as shown by increased incidences of renal tubule adenomas. Thyroid follicular cell neoplasms and mononuclear cell leukemia in male and female rats may have been related to chemical administration. There was *equivocal evidence of carcinogenic activity* for male $B6C3F_1$ mice as shown by a marginally increased incidence of renal tubule cell neoplasms. There was *equivocal evidence of carcinogenic activity* for female $B6C3F_1$ mice as shown by a marginally increased incidence of harderian gland adenomas.

Renal tubule cell hyperplasia in male and female rats and gliosis, hemorrhage, pigmentation (hemosiderin accumulation), and mineralization in the brains of female rats were associated with the administration of tris(2-chloroethyl) phosphate. Karyomegaly of renal tubule epithelial cells in male and female mice was also chemical related.

Synonyms: 2-chloroethanol phosphate (3:1); tris(β-chloroethyl) phosphate

Trade Names: Fyrol CEF; Disflamoll TCA; NIAX flame retardant

Report Date: May 1991

TR-392 Toxicology and Carcinogenesis Studies of Chlorinated Water (CAS Nos. 7782-50-5 and 7681-52-9) and Chloraminated Water (CAS No. 10599-90-3) (Deionized and Charcoal-Filtered) in F344/N Rats and $B6C3F_1$ Mice (Drinking Water Studies)

Chlorine and chloramine are used as disinfectants in water supplies to prevent the spread of waterborne diseases. The U.S. Environmental Protection Agency and the U.S. Congress, through the Safe Drinking Water Acts and Amendments, initiated studies to determine the most effective way to disinfect water supplies and, at the same time, minimize any potential long-term health effects associated with direct chemical exposure or indirect chemical exposure through the formation of byproducts. As part of this evaluation, 2-year studies of chlorinated or chloraminated deionized charcoal-filtered drinking water were conducted in F344/N rats and $B6C3F_1$ mice to determine the potential toxicity and carcinogenicity associated with prolonged exposure and eliminate possible confounding effects of byproducts of chlorination.

Chlorinated Water Studies: Water containing 0, 70, 140, or 275 ppm chlorine (based on available atomic chlorine) was provided to groups of 70 F344/N rats or $B6C3F_1$ mice of each sex for up to 2 years. Groups of 10 rats or mice of each sex were predesignated for evaluation at 14 or 15 weeks and 66 weeks. Survival at 2 years of rats and mice receiving chlorinated water was similar to that of the controls. Mean body weights of dosed male rats, high-dose female rats, and dosed mice were slightly lower than those of their respective control groups. There was a dose-related decrease in water consumption by rats and mice. Water consumption by high-dose rats during the second year of the studies was 21% lower than

controls for males and 23% lower than controls for females; water consumption by high-dose mice was 31% lower than controls for males and 26% lower than controls for females.

The incidence of mononuclear cell leukemia in mid-dose, but not high-dose, female rats was significantly higher than that in controls (control, 8/50; low-dose, 7/50; mid-dose, 19/51; high-dose, 16/50). The proportion of female rats that died of leukemia before the end of the study and the mean time for observation of animals dying with leukemia were similar among all dose groups and controls. Although the marginal increase in leukemia incidence in the mid- and high-dose female rats suggested a possible association with the administration of chlorinated water, the incidence of leukemia was not clearly dose related. There was no indication of reduced latency of leukemia, and the incidence of leukemia in concurrent controls was less than the mean for historical controls; furthermore, there was no supporting evidence of an effect in male rats. Thus, the marginal increase in leukemia incidence in female rats was considered equivocal evidence of carcinogenic activity. There were no neoplasms or nonneoplastic lesions in male rats or in male or female mice that were clearly associated with the consumption of chlorinated water.

Chloraminated Water Studies: Water containing 50, 100, or 200 ppm chloramine was provided to groups of 70 F344/N rats or $B6C3F_1$ mice of each sex for up to 2 years. The same control groups were used for the chlorinated water and chloraminated water studies. Groups of 9 or 10 rats or mice of each sex were evaluated at 14 or 15 weeks and 66 weeks.

Survival at 2 years of rats and mice receiving chloraminated water was similar to that of the controls. Mean body weights of high-dose rats and dosed mice were lower than those of their respective control groups. There was a dose-related decrease in water consumption by rats and mice. Water consumption during the second year of the studies by high-dose rats was 34% lower than controls for males and 31% lower than controls for females; water consumption by high-dose mice was 42% lower than controls for males and 40% lower than controls for females.

Mononuclear cell leukemia occurred with a marginally increased incidence in the mid- and high-dose female rats receiving chloraminated water (control, 8/50; low dose, 11/50; mid dose, 15/50; and high dose, 16/50). As in female rats receiving chlorinated water, the proportion of female rats that died of leukemia before the end of the study and the mean time for observation of animals dying with leukemia were similar among all dose groups and controls. The marginal increase in leukemia incidence in females receiving chloraminated water was considered equivocal evidence of carcinogenic activity for the same reasons given for female rats receiving chlorinated water. There were no neoplasms or nonneoplastic lesions in male rats or in male or female mice that were clearly associated with the consumption of chloraminated water.

Conclusions: Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity* of chlorinated water in male F344/N rats receiving 70, 140, or 275 ppm. There was *equivocal evidence of carcinogenic activity* of chlorinated water in female F344/N rats based on an increase in the incidence of mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of chlorinated water in male or female $B6C3F_1$ mice receiving 70, 140, or 275 ppm.

Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity* of chloraminated water in male F344/N rats receiving 50, 100, or 200 ppm. There was *equivocal evidence of carcinogenic activity* of chloraminated water in female F344/N rats based on an increase in the incidence of mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of chloraminated water in male or female $B6C3F_1$ mice receiving 50, 100, or 200 ppm.

Report Date: March 1992

TR-393 Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and $B6C3F_1$ Mice (Drinking Water Studies)

Sodium fluoride is a white, crystalline, water-soluble powder used in municipal water fluoridation systems, in various dental products, and in a variety of industrial applications. Toxicology and carcinogenesis studies were conducted with F344/N rats and $B6C3F_1$ mice of each sex by incorporating sodium fluoride into the drinking water in studies lasting 14 days, 6 months, and 2 years. In addition, genetic toxicology studies were performed with *Salmonella typhimurium*, with mouse L5178Y cells, and with Chinese hamster ovary cells.

14-Day Studies: Rats and mice received sodium fluoride in drinking water at concentrations as high as 800 ppm. (Concentrations are expressed as sodium fluoride; fluoride ion is 45% of the sodium salt by weight.) In the high-dose groups, 5/5 male and 5/5 female rats and 2/5 male mice died; one female rat was given 400 ppm in the drinking water also died before the end of the studies. No gross lesions were attributed to sodium fluoride administration.

6-Month Studies: Rats received concentrations of sodium fluoride in drinking water as high as 300 ppm, and mice as high as 600 ppm. No rats died during the studies; however, among the mice, 4/9 high-dose males, 9/11 high-dose females, and 1/8 males in the 300 ppm group died before the end of the studies. Weight gains were less than those of controls for rats receiving 300 ppm and mice receiving 200 to 600 ppm.

The teeth of rats and mice receiving the higher doses of sodium fluoride were chalky white and chipped or showed unusual wear patterns. Mice and male rats given the higher concentrations had microscopic focal degeneration of the enamel organ. Rats receiving 100 or 300 ppm sodium fluoride had minimal hyperplasia of the gastric

mucosa of the stomach, and one high-dose rat of each sex had an ulcer. Acute nephrosis and/or lesions in the liver and myocardium were observed in mice that died early, and minimal alterations in bone growth/remodeling were observed in the long bones of mice receiving sodium fluoride at concentrations of 50 to 600 ppm.

The sodium fluoride concentrations selected for the 2-year studies in both rats and mice were 0, 25, 100, and 175 ppm in the drinking water. These concentrations were selected based on the decreased weight gain of rats at 300 ppm and of mice at 200 ppm and above, on the incidence of gastric lesions in rats at 300 ppm in the 6-month studies, and on the absence of significant toxic effects at sodium fluoride concentrations as high as 100 ppm in an earlier 2-year study.

Body Weights and Survival in the 2-Year Studies: Mean body weights of dosed and control groups of rats and mice were similar throughout the 2-year studies. Survival of rats and mice was not affected by sodium fluoride administration. Survival rates after 2 years were: male rats-control, 42/80; 25 ppm, 25/51; 100 ppm, 23/50; 175 ppm, 42/80; female rats-59/80; 31/50; 34/50; 54/81; male mice-58/79; 39/50; 37/51; 65/80; female mice-53/80; 38/52; 34/50; 52/80.

Neoplastic and Nonneoplastic Effects in the 2-Year Studies: The teeth of rats and mice has a dose-dependent whitish discoloration, and male rats had an increased incidence of tooth deformities and attrition leading on occasion to malocclusion. The teeth of male and, to a lesser degree, female rats had areas of microscopic dentine dysplasia and degeneration of ameloblasts. Dentine dysplasia occurred in both dosed and control groups of male and female mice; the incidence of this lesion was significantly greater in high-dose than in control male mice. Osteosclerosis of long bones was increased in female rats given drinking water containing 175 ppm sodium fluoride. No other significant nonneoplastic lesions in rats or mice appeared related to sodium fluoride administration.

Osteosarcomas of bone were observed in 1/50 male rats in the 100 ppm group and in 3/80 male rats in the 175 ppm group. None were seen in the control or 25 ppm dose groups. One other 175 ppm male rat had an extraskeletal osteosarcoma arising in the subcutaneous tissue. Osteosarcomas occur in historical control male rats at an incidence of 0.5% (range 0-6%). The historical incidence is not directly comparable with the incidences observed in this study because examination of bone was more comprehensive in the sodium fluoride studies than in previous NTP studies of other chemicals, and the diet used in previous studies was not controlled for fluoride content. In the current study, although the pairwise comparison of the incidence in the 175 ppm group versus that in the controls was not statistically significant, osteosarcomas occurred with a statistically significant dose-response trend, leading to the conclusion that a weak association may exist between the occurrence of these neoplasms and the administration of sodium fluoride. No other neoplastic lesions in rats or mice were considered possibly related to chemical administration.

Genetic Toxicology: Sodium fluoride was negative for gene mutation induction in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with and without S9. In two laboratories, sodium fluoride was tested for induction of trifluorothymidine resistance in mouse L5178Y lymphoma cells; results were positive both with and without S9. Sodium fluoride was tested for cytogenetic effects in Chinese hamster ovary (CHO) cells in two laboratories. In the first laboratory, the sister chromatid exchange (SCE) test was negative with and without S9, and the chromosomal aberration (Abs) test was positive in the absence of S9; in the second laboratory, the SCE test was positive with and without S9, but no induction of Abs was observed. The laboratory that reported a negative result for Abs tested at doses below that shown to be positive at the other laboratory. Similarly, the positive SCE result was obtained at a higher dose and longer harvest time than used by the laboratory reporting the negative SCE response.

Conclusions: Under the conditions of these 2-year dosed water studies, there was *equivocal evidence of carcinogenic activity* of sodium fluoride in male F344/N rats, based on the occurrence of a small number of osteosarcomas in dosed animals. "Equivocal evidence" is a category for uncertain findings defined as studies that are interpreted as showing a marginal increase of neoplasms that may be related to chemical administration. There was *no evidence of carcinogenic activity* in female F344/N rats receiving sodium fluoride at concentrations of 25, 100, or 175 ppm (11, 45, or 79 ppm fluoride) in drinking water for 2 years. There was *no evidence of carcinogenic activity* of sodium fluoride in male or female mice receiving sodium fluoride at concentrations of 25, 100, or 175 ppm in drinking water for 2 years.

Dosed rats had lesions typical of fluorosis of the teeth and female rats receiving drinking water containing 175 ppm sodium fluoride had increased osteosclerosis of long bones.

Report Date: December 1990

TR-394 Toxicology and Carcinogenesis Studies of Acetaminophen (CAS No. 103-90-2) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-395 Toxicology and Carcinogenesis Studies of Probenecid (CAS No. 57-66-9) in F344/N Rats and B6C3F$_1$ (Gavage Studies)

Probenecid is a white crystalline solid commonly used as a uricosuric agent in the treatment of gout. Because of its inhibitory effects on renal tubule transport processes,

probenecid is also used as a therapeutic adjunct to enhance blood levels of penicillin and its action. Toxicology and carcinogenicity studies were conducted by administering probenecid (>99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex once daily, 5 days per week in 14-day, 13-week, and 2-year studies. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary cells.

14-Day Studies: Doses used in the 14-day studies for both rats and mice were 0, 200, 400, 800, 1,600, or 3,200 mg/kg. Of the animals receiving 3,200 mg/kg, all rats, all female mice, and two of five male mice died during the studies. No deaths occurred among the other dose groups. There was a significant reduction in body weight gain in male and female rats receiving 1,600 mg/kg and in female rats receiving 800 mg/kg. No gross lesions were attributed to probenecid administration in rats or mice of either sex.

13-Week Studies: Doses used in the 13-week studies were 0, 50, 100, 200, 400, or 800 mg/kg for rats and 0, 100, 200, 400, 800, or 1,600 mg/kg for mice. No rats died during the 13-week studies. In mice, 5 of 10 males and 3 of 10 females receiving 1,600 mg/kg and 1 of 10 males receiving 800 mg/kg died during the study. Significant reductions in body weight gain occurred in male rats administered 800 mg/kg, male mice administered 1,600 mg/kg, and female mice administered 800 or 1,600 mg/kg. All dose groups of male rats and all groups of female rats receiving 100 mg/kg or more showed significant increases in absolute and/or relative liver weights compared to control groups. This change was also seen in mice receiving 200 mg/kg and greater, except female mice in the 400 mg/kg group. No compound-related lesions occurred in rats or mice of either sex.

Based on compound-related deaths and suppression of body weight gains observed at higher doses in the 13-week studies, doses of 0, 100, and 400 mg/kg were used for the 2-year studies in rats and mice. These doses were administered once daily, 5 days a week for up to 103 weeks to groups of 50 males or 50 females of each species.

Body Weight and Survival in the 2-Year Studies: The mean body weight of high-dose female rats was 10% to 20% lower than that of controls throughout the studies. Mean body weights for all other dosed rats and for all dosed mice were similar to those of controls throughout the 2-year studies.

Survival of high-dose male rats and high-dose and low-dose male mice was significantly lower than that of controls. Survival rates after 2 years were: male rats—control, 37/50; 100 mg/kg, 34/50; 400 mg/kg, 22/50; female rats—24/50; 35/50; 19/50; male mice—38/50; 23/50; 24/50; female mice—32/49; 32/49; 32/50.

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: No chemical-related histopathologic toxic effects or increased incidence of tumors attributable to probenecid were observed in male or female rats receiving probenecid by corn oil gavage for up to 2 years. Mammary gland fibroadenomas and combined thyroid C-cell adenomas or carcinomas exhibited significant negative trends in female rats. These decreased tumor rates were associated with lower body weights. The incidence of adrenal medullary pheochromocytomas was significantly decreased in high-dose male rats. No compound-related increase in nonneoplastic lesions was observed in rats of either sex.

No compound-related neoplastic effects were observed in male mice. In high-dose female mice, there were significant increases in the incidences of hepatocellular adenomas (3/48; 2/49; 14/49), but there was no corresponding increase in carcinomas (2/48; 2/49; 3/49). Treatment-related increased incidences of ovarian abscesses in female mice were causally related to *Klebsiella* species infection rather than directly related to chemical administration.

Genetic Toxicology : Probenecid was not mutagenic in *Salmonella typhimurium* strain TA100, TA1535, TA1537, or TA98 with or without metabolic activation. In cytogenetic tests with Chinese hamster ovary cells, probenecid induced sister chromatid exchanges in the absence, but not in the presence of S9 activation. No induction of chromosomal aberrations was observed with or without S9.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of probenecid for male or female F344/N rats receiving 100 or 400 mg/kg in corn oil. There was *no evidence of carcinogenic activity* of probenecid for male B6C3F$_1$ mice given 100 or 400 mg/kg probenecid in corn oil. There was *some evidence of carcinogenic activity* of probenecid for female B6C3F$_1$ mice based on an increased incidence of hepatocellular adenomas.

Synonyms: 4-[(Dipropylamino)sulfonyl]benzoic acid; *p*-(dipropylsulfamoyl)benzoic acid; *p*-(dipropylsulfamyl)benzoic acid

Trade Names: Benacen; Benemid; Benemide; Benn; Probalan; Probecid; Proben; Probenid; Robenecid; Uricocid

Report Date: September 1991

TR-396 Toxicology and Carcinogenesis Studies of Monochloroacetic Acid (CAS No. 79-11-8) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Monochloroacetic acid, a colorless crystalline material, is used as a postemergence contact herbicide and as an intermediate in the synthesis of other organic compounds. Toxicology and carcinogenicity studies were conducted by administering monochloroacetic acid (99% pure) in deionized water by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex once daily, 5 days per week for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma L5178Y cells, Chinese hamster ovary cells, and *Drosophila melanogaster*.

16-Day Studies: Groups of five rats of each sex received 0, 7.5, 15, 30, 60, or 120 mg monochloroacetic acid/kg body

weight. Doses administered to mice were 0, 15, 30, 60, 120, or 240 mg/kg to groups of five males and 0, 30, 60, 120, 240, or 480 mg/kg to groups of five females. One of five male rats given 120 mg/kg died during the studies. Clear nasal discharge, lacrimation, or both, were observed in all groups of male and female rats receiving monochloroacetic acid. No compound-related gross lesions were observed in rats. All male mice given 240 mg/kg and all females given 240 or 480 mg/kg died during the studies. Hypoactivity, piloerection, ataxia, and lacrimation were observed in mice given 240 or 480 mg/kg. No compound-related gross lesions were observed in mice at necropsy.

13-Week Studies: Groups of 20 rats of each sex received 0, 30, 60, 90, 120, or 150 mg/kg monochloroacetic acid, and groups of 20 mice of each sex received doses of 0, 25, 50, 100, 150, or 200 mg/kg. Three to five animals in each dose group were killed at weeks 4 and 8 for the evaluation of hematology parameters. Compound-related deaths occurred in rats in the three highest dose groups (all males given 120 or 150 mg/kg, 9/10 males given 90 mg/kg, and all females given 90 to 150 mg/kg) and in mice given 200 mg/kg (all males and 2/10 females). Final mean body weights of surviving rats and mice receiving monochloroacetic acid were similar to those of controls. In rats, dose-related increases in blood urea nitrogen, alanine aminotransferase, and aspartate aminotransferase levels were observed, and relative liver and kidney weights were elevated. There were no compound-related changes in the various hematologic or clinical pathology parameters in mice. A dose-related increase in the incidence and severity of cardiomyopathy was observed in male and female rats receiving monochloroacetic acid, and hepatocellular cytoplasmic vacuolization was observed in the high-dose mice that died during the studies.

2-Year Studies: Based on the mortality and compound-related histopathologic lesions observed in the 13-week studies, doses selected for the 2-year studies of monochloroacetic acid were 0, 15, or 30 mg/kg, administered to groups of 70 rats of each sex, and 0, 50, or 100 mg/kg, administered to groups of 60 mice of each sex. Interim evaluations were conducted on 10 rats per dose group after 6 months of treatment with monochloroacetic acid and on seven rats per dose group after 15 months of treatment.

Body Weight and Survival in the 2-Year Studies: Mean body weights of low- and high-dose female and low-dose male rats receiving monochloroacetic acid were within 10% of those of controls throughout the studies; however, after week 30, the mean body weights of high-dose male rats were 4% to 8% less than those of controls. In mice, the mean body weights of dosed males were similar to controls, but those of low- and high-dose females were 6% to 10% less than control values after week 52. Survival of high-dose male and dosed female rats and high-dose male mice was significantly lower than that of controls (male rats: control, 27/53; low-dose, 21/53; high-dose, 16/53; female rats: 37/53; 19/53; 26/53; male mice: 46/60; 39/60; 21/60; female mice: 42/60; 40/60; 44/60).

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: There was no compound-related increase in the incidence of neoplasms or nonneoplastic lesions in rats given monochloroacetic acid for 2 years. The incidence of uterine stromal polyps in low- and high-dose female rats was slightly higher than that in controls (2/60; 7/57; 10/60). However, the incidence in the controls was unusually low, and those in the dosed groups were well within the range for NTP historical controls (mean: 21%, range: 10%-38%). Further, because the only malignant stromal neoplasm occurred in a control animal, the polyps were not considered to be related to the administration of monochloroacetic acid. Similarly, there was no monochloroacetic acid-related increase in the incidence of neoplasms in male or female mice, and malignant lymphoma occurred with a significant negative trend in dosed female mice. Increases in the incidence of inflammation of the mucosa of the nasal passages, respiratory epithelial metaplasia of the olfactory epithelium of the nose, and focal squamous cell hyperplasia of the forestomach occurred in dosed male and female mice.

Genetic Toxicology: Monochloroacetic acid was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98, with or without exogenous metabolic activation (S9). It induced trifluorothymidine resistance in L5178Y cells in the absence of S9 and induced sister chromatid exchanges without S9 in Chinese hamster ovary cells. Monochloroacetic acid did not induce a significant increase in chromosomal aberrations in Chinese hamster ovary cells, with or without S9. Monochloroacetic acid administered in feed was negative for the induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster;* however, when it was administered by injection, the results were equivocal.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* for monochloroacetic acid in male or female F344/N rats given 15 or 30 mg/kg. There was *no evidence of carcinogenic activity* for monochloroacetic acid in male or female B6C3F_1 mice given 50 or 100 mg/kg.

Monochloroacetic acid administration was associated with inflammatory lesions of the nasal mucosa, metaplasia of the olfactory epithelium, and squamous cell hyperplasia of the forestomach in male and female mice.

Synonyms: Chloroacetic acid, α-chloroacetic acid, chloroethanoic acid

Report Date: January 1992

TR-397 Toxicology and Carcinogenesis Studies of C.I. Direct Blue 15 (CAS No. 2429-74-5) in F344 Rats (Drinking Water Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-398 Toxicology and Carcinogenesis Studies of Polybrominated Biphenyl Mixture (Firemaster FF-1) (CAS No. 67774-32-7) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

Note: Polybrominated Biphenyl Mixture (Firemaster FF-1) was previously tested in F344 rats and B6C3F$_1$ mice administered in feed (See TR-244, reported 1983).

TR-399 Toxicology and Carcinogenesis Studies of Titanocene Dichloride (CAS No. 1271-19-8) in F344/N Rats (Gavage Studies)

Titanocene dichloride is an organometallic compound composed of two cyclopentadienyl rings, titanium, and chloride. It is used as a cocatalyst in polymerization reactions. Toxicology and carcinogenesis studies were conducted by administering titanocene dichloride (greater than 98% pure) in corn oil by gavage to groups of F344/N rats for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and in Chinese hamster ovary cells.

14-Day and 13-Week Studies: In the 14-day studies, titanocene dichloride was administered at doses of 0, 65, 125, 250, 500, or 1,000 mg/kg. All high-dose rats and four of the five male and two of the five female rats given 500 mg/kg died during the studies. A dose-related decrease in body weight gain was seen in rats given 125, 250, 500, and 1,000 mg/kg. Lesions related to chemical administration included hepatocellular necrosis, tubule necrosis in the kidney, erosions and ulcers of the glandular stomach, and hyperplasia of the forestomach epithelium.

The 13-week studies were conducted by administering titanocene dichloride at doses of 0, 8, 16, 31, 62, or 125 mg/kg. One female rat in the 125 mg/kg dose group died from chemical toxicity during the fourth week of the studies. Body weight gain was lower in rats given 62 or 125 mg/kg than in control groups. Treatment-associated histopathologic lesions were seen in the stomachs of high-dose males and all groups of females given titanocene dichloride. These lesions included hyperplasia and metaplasia of the glandular stomach and hyperplasia and hyperkeratosis of the forestomach.

Body Weight and Survival in the 2-Year Studies: The doses selected for the 2-year studies in rats (0, 25, and 50 mg/kg) were based on the potentially life-threatening nature of the glandular stomach lesions and the decreased body weight gain compared to controls seen in the 62 and 125 mg/kg dose groups in the 13-week studies.

The final mean body weights of high-dose males and females were 91% and 89% of controls, respectively. The 2-year survival rates for males in the control, low-, and high-dose groups were 41/60, 30/60, and 24/60; survival rates for female rats were 37/60, 30/61, and 31/60.

Nonneoplastic and Neoplastic Effects in the 2-Year Studies: The principal toxic effects associated with the administration of titanocene dichloride for 2 years occurred in the stomach. The lesions in the stomach were seen at the 15-month interim evaluations and were similar to, but less severe than, those observed at 2 years. The lesions included focal erosions of the glandular mucosa with an associated inflammatory response, hyperplasia and metaplasia of the epithelium of the fundic glands, and fibrosis of the lamina propria and submucosa. Forestomach lesions included focal acanthosis (hyperplasia) and hyperkeratosis of the stratified squamous epithelium. Squamous cell papillomas of the forestomach were seen in four low-dose males, one high-dose male, one low-dose female, and two high-dose females; none were observed in controls. A squamous cell carcinoma of the forestomach occurred in one low-dose male and a benign basosquamous tumor occurred in one high-dose male.

Accumulations of macrophages with blue-gray pigment believed to contain titanium were present in many organs of dosed rats including the gastrointestinal tract, liver, lung, and lymph nodes. A dose-related increase in the incidence of inflammation of the nasal mucosa and lung also occurred and was attributed to reflux and/or regurgitation and aspiration of gavage solution due to the severe stomach lesions.

Genetic Toxicology: Titanocene dichloride was mutagenic in *Salmonella typhimurium* strain TA100 in the absence of exogenous metabolic activation (S9); it was not mutagenic in TA100 with S9, nor was it mutagenic in TA1535, TA1537, or TA98 with or without S9. Titanocene dichloride did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells, with or without S9.

Conclusions: Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity* of titanocene dichloride in male F344/N rats based on a marginal increase in the incidence of forestomach squamous cell papillomas, squamous cell carcinoma, and basosquamous tumor benign. There was *equivocal evidence of carcinogenic activity* of titanocene dichloride in female F344/N rats based on a marginal increase in the incidence of forestomach squamous cell papillomas.

Nonneoplastic lesions associated with the administration of titanocene dichloride for up to 2 years included erosions and inflammation of the gastric mucosa, hyperplasia and metaplasia of the fundic glands with fibrosis of the lamina propria in the glandular stomach, and acanthosis (hyperplasia) and hyperkeratosis of the forestomach epithelium.

Synonyms: Titanium ferrocene; biscyclopentadienyltitanium dichloride; dichlorodi-π-cyclopentadienyltitanium; dichlorobis(η^5-2,4-cyclopentadien-1-yl)titanium; dicyclopentadienyltitanium dichloride; dichlorodicyclopentadienyltitanium; dichlorotitanocene; dicyclopentadi-

enyldichlorotitanium; dichlorobis(π-cyclopentadienyl)-titanium; bis(η^5-cyclopentadienyl)titanium dichloride; dichlorobis(η^5-cyclopentadienyl)titanium; dichlorobis-cyclopentadienyl titanium; dichlorobis(1,3-cyclopentadiene)titanium; bis(cyclopentadienyl)dichlorotitanium

Report Date: September 1991

TR-400 Toxicology and Carcinogenesis Studies of 2,3-Dibromo-1-Propanol (CAS No. 96-13-9) in F344 Rats and B6C3F$_1$ Mice (Dermal Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-401 Toxicology and Carcinogenesis Studies of 2,4-Dirminophenol Dihydrochloride (CAS No. 137-09-7) in F344 Rats and B6C3F$_1$ Mice (Gavage Studies

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-402 Toxicology and Carcinogenesis Studies of Furan (CAS No. 110-00-9) in F344 Rats and B6C3F$_1$ Mice (Gavage Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-403 Toxicology and Carcinogenesis Studies of Resorcinol (CAS No. 108-46-3) in F344 Rats and B6C3F$_1$ Mice (Gavage Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-404 Toxicology and Carcinogenesis Studies of Diphenylhydantoin (Phenytoin) (CAS No. 57-41-0) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-405 Toxicology and Carcinogenesis Studies of C.I. Acid Red 114 (CAS No. 6459-94-5) in F344/N Rats (Drinking Water Studies)

C.I. Acid Red 114 is one of five chemicals being evaluated in 2-year carcinogenicity and toxicity studies as part of the NTP's Benzidine Dye Initiative. This Initiative was designed to evaluate representative benzidine congeners, benzidine congener-derived dyes, and benzidine-derived dyes. C.I. Acid Red 114 was nominated for study because of the potential for human exposure during production of bisazobiphenyl dyes and because benzidine, a structurally related chemical, is a known human carcinogen.

Toxicology and carcinogenesis studies were conducted by administering desalted, industrial grade C.I. Acid Red 114 in drinking water to groups of F344/N rats of each sex for 13 days, 13 weeks, 9 or 15 months, or 2 years. These studies were performed only in rats because studies of benzidine congeners were being performed in mice at the National Center for Toxicological Research (NCTR). Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary cells, and *Drosophila melanogaster*.

13-Day Studies: Rats were exposed to C.I. Acid Red 114 in drinking water at doses of 0, 10,000, 20,000, or 30,000 ppm. All control and dosed rats survived except one male rat in the 20,000 ppm dose group. Final mean body weights in the three dosed groups were 94%, 83%, or 77% of controls for males and 92%, 88%, or 80% of controls for females. Water consumption declined with increased dose. Clinical findings included red stained fur, ears, and tail in all test animals. On gross necropsy, organs and tissues were also stained red.

13-Week Studies: C.I. Acid Red 114 was administered in drinking water at doses of 0, 600, 1,200, 2,500, 5,000, or 10,000 ppm. All control and dosed animals survived until the end of the study. Final mean body weights in the five dosed groups were 97%, 89%, 87%, 87%, or 85% of controls for males and 97%, 94%, 94%, 92%, or 89% of controls for females. Water consumption was decreased in dosed animals. As was seen in the 13-day studies, major organs and tissues from treated animals were stained red. Kidney toxicity characterized by regeneration and karyomegaly of tubule epithelial cells with chronic inflammation was observed in female rats at doses of 1,200 ppm or above. Treatment-related increases in relative liver weights and elevated liver enzyme levels were seen in males and females, centrilobular pallor in the liver was seen in all male dose groups. Because of these body weight differences, decreases in water consumption, and organ toxicity, the doses chosen for the 2-year studies were 70, 150, and 300 ppm for males and 150, 300, and 600 for females.

2-Year Studies: Male rats received doses of 0, 70, 150, or 300 ppm of C.I. Acid Red 114, and female rats received 0, 150, 300, or 600 ppm. Seventy animals were in the control and high-dose groups, 45 in the low-dose groups,

and 75 in the mid-dose groups. Ten animals were evaluated from the control and high-dose groups at 9 months, and ten animals from all dose groups were evaluated at 15 months. The average amount of compound consumed per day was 4, 8, or 20 mg/kg for males and 9, 20, or 70 mg/kg for females.

Survival and Body Weights: Survival at 105 weeks for male rats receiving 0, 70, 150, or 300 ppm was 24/50, 15/35, 26/65, and 1/50; for females receiving 0, 150, or 300 ppm, survival was 36/50, 13/35, and 6/64. All female rats receiving 600 ppm died by week 89. The decreased survival in treated groups was due primarily to the development of chemical-related neoplasms. Of the surviving animals, the final mean body weights for males receiving 70 or 150 ppm were 94% and 90% of control and for females receiving 150 or 300 ppm, 99% and 84% of control. These weight differences began in the second year of the studies and were attributed in part to the development of neoplasms in the dosed groups.

Histopathologic Effects in the 2-Year Studies: At 9 and 15 months, a few neoplasms were seen in the liver, lung, clitoral gland, skin, Zymbal's gland, oral cavity epithelium, and small and large intestine, and the number of neoplasms at these sites increased as the studies progressed. At 2 years, there was a clear carcinogenic response in the skin, Zymbal's gland, and liver of male and female rats, and in the clitoral gland, oral cavity epithelium, small and large intestine, and lung in female rats. Treatment-related increases were also seen in the incidence in neoplasms of the oral cavity epithelium, adrenal gland, and lung of male rats, and in mononuclear cell leukemia and in neoplasms of the mammary gland and adrenal gland in female rats. The incidence of these neoplasms was generally lower, but was significant and considered to be marginally related to chemical treatment. The same neoplastic effects have been previously observed in some or all of the NTP studies with dimethoxybenzidine, dimethylbenzidine, or C.I. Direct Blue 15.

Genetic Toxicology: In a standard preincubation protocol, C.I. Acid Red 114 was mutagenic in *Salmonella typhimurium* strain TA98 in the presence of induced hamster liver S9, and an equivocal response was noted in strain TA100 with hamster liver S9. However, no significant mutagenic activity was noted in strains TA1535 or TA1537 with or without S9 activation. In a modified *S. typhimurium* gene mutation test which employed reductive metabolism followed by oxidative metabolism with S9 liver enzymes, C.I. Acid Red 114 was strongly mutagenic in strain TA1538. C.I. Acid Red 114 did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells with or without S9 activation; reductive metabolism was not used in these cytogenetic tests. No increase in sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* administered C.I. Acid Red 114 by feeding or injection.

Conclusions: Under the conditions of these 2-year drinking water studies, there was *clear evidence of carcinogenic activity* of C.I. Acid Red 114 for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, and liver. Increased incidences of neoplasms of the oral cavity epithelium, adrenal gland, and lung may have been related to chemical administration. There was *clear evidence of carcinogenic activity* for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity epithelium, small and large intestines, and lung. Increased incidences of mononuclear cell leukemia, mammary gland adenocarcinoma, and adrenal gland pheochromocytomas may have been related to chemical administration.

Synonyms: 1,3-Naphthalenedisulfonic acid, 8-((3,3'-dimethyl-4'-((4-(((4-methylphenyl)sulfonyl) oxy)phenyl) azo) (1,1'-biphenyl)-4-yl)azo)-7-hydroxy, disodium salt, Acid Leather Red BG, Acid Red 114, Amacid Milling Red PRS, Benzyl Fast Red BG, Benzyl Red BR, Cerven Kysela, C.I. 23635, Erionyl Red RS, Folan Red B, Kayanol Milling Red RS, Leather Fast Red B, Levanol Red GG, Midlon Red PRS, Milling Red B, Milling Red BB, Milling Red SWB, NCI C61096, Polar Red RS, Sandolan Red N-RS, Sella Fast Red RS, Sulphonol Fast Red R, Supranol Fast Red GG, Supranol Red PBX-CF, Supranol Red R, Telon Fast Red GG, Tertracid Milling Red B, Vondamol Fast Red RS

Report Date: December 1991

TR-406 Toxicology and Carcinogenesis Studies of γ-Butyrolactone (CAS No. 96-48-0) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

γ-Butyrolactone is an intermediate in the synthesis of polymers used as film formers in hair sprays, blood plasma extenders, and clarifying agents in beer and wine. Toxicology and carcinogenesis studies were conducted by administering γ-butyrolactone (greater than 97% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex, 5 days per week for 16 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Drosophila melanogaster*, and Chinese hamster ovary cells.

16-Day Studies: Groups of five rats of each sex received doses of 0, 75, 150, 300, 600, or 1,200 mg of γ-butyrolactone per kg of body weight and groups of five mice of each sex received doses of 0, 87, 175, 350, 700, or 1,400 mg/kg. All male and female rats given 1,200 mg/kg and one male rat given 600 mg/kg died within 3 days. The mean body weight gain of female rats given 600 mg/kg was significantly lower than that of the controls. Mean body weight gains of the other female dose groups and all male dose groups were similar to those of the controls. All of the male and four female mice receiving 1,400 mg/kg died during the studies. Mean body weight gains of dosed mice were generally similar to those of the controls. Rats

receiving 600 or 1,200 mg/kg and mice receiving 350 mg/kg or more became inactive or recumbent with irregular respiration following dosing.

13-Week Studies: Groups of 10 rats of each sex received doses of 0, 56, 112, 225, 450, or 900 mg of γ-butyrolactone per kg of body weight and groups of 10 mice of each sex received doses of 0, 65, 131, 262, 525, or 1,050 mg/kg. One female and all male rats given 900 mg/kg died during the studies. The final mean body weight and mean body weight gain of male rats receiving 450 mg/kg were significantly lower than those of the controls; final mean body weights and body weight gains of all female rat dose groups were similar to those of the controls. There was an increased incidence of focal inflammation of the nasal mucosa in rats administered γ-butyrolactone. Three male mice and one female receiving 1,050 mg/kg died from γ-butyrolactone toxicity during the studies. The mean body weight gain and final mean body weight of high-dose male mice were lower than those of the controls; the mean body weight gains and final mean body weights of dosed female mice were similar to those of the controls. No lesions related to the administration of γ-butyrolactone occurred in mice of either sex.

2-Year Studies: The doses administered to groups of 50 animals per sex were 0, 112, and 225 mg of γ-butyrolactone per kg of body weight for male rats; 0, 225, and 450 mg/kg for female rats; and 0, 262, and 525 mg/kg for male and female mice.

Body Weight and Survival in the 2-Year Studies: The mean body weights of male rats administered γ-butyrolactone were similar to those of the controls throughout the study. The mean body weight of high-dose females was lower than that of the controls after week 5 and was 10% to 20% lower than that of the controls throughout the second year. The survival of high-dose male rats was slightly higher than that of the controls (control, 24/50; low-dose, 27/50, high-dose, 32/50) due primarily to a lower incidence of mononuclear cell leukemia in the high-dose group (16/50, 15/50, 9/50). The survival of dosed females was similar to that of the controls (28/50, 27/50, 28/50).

The mean body weights of dosed male mice were lower than those of the controls throughout the study, but the differences in mean body weights decreased when male mice were housed individually at week 67. The final mean body weights of dosed male mice were 6% lower than that of the controls. Mean body weights of dosed female mice were also lower than those of the controls throughout the study, and the final mean body weights were from 14% to 17% lower than that of the controls. The survival in high-dose male mice was significantly lower than that of the controls (35/50, 30/50, 12/50) due to bite wounds and fighting in high-dose males recovering from the sedative effects of γ-butyrolactone. The survival of female dosed mice was similar to that of the controls (38/50, 34/50, 38/50).

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: No increased incidences of neoplasms or nonneoplastic lesions in male rats were related to the administration of γ-butyrolactone for 2 years. In female rats, negative trends were observed in the incidences of cysts (42/50, 35/50, 23/50) and fibroadenomas of the mammary gland (22/50, 14/50, 6/50) and in cysts of the pituitary pars distalis (25/49, 13/37, 11/48). These decreases were considered to be related to γ-butyrolactone administration.

Increased incidences of proliferative lesions, primarily hyperplasia, of the adrenal medulla in low-dose male mice were associated with γ-butyrolactone administration (pheochromocytoma, benign or malignant: 2/48, 6/50, 1/50; hyperplasia: 2/48, 9/50, 4/50). The incidence of hepatocellular neoplasms in both dose groups of male mice was lower than the incidence in the controls (hepatocellular adenoma or carcinoma: 24/50, 8/50, 9/50).

Genetic Toxicology: γ-Butyrolactone was not mutagenic, with or without exogenous metabolic activation (S9), in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, nor did it induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered in feed or by injection. Positive results were obtained, however, in cytogenetic tests with Chinese hamster ovary cells; γ-butyrolactone induced sister chromatid exchanges and chromosomal aberrations in trials conducted in the presence of S9 activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of γ-butyrolactone in male F344/N rats given 112 or 225 mg/kg or in female F344/N rats given 225 or 450 mg/kg in corn oil. There was *equivocal evidence of carcinogenic activity* of γ-butyrolactone in male $B6C3F_1$ mice based on marginally increased incidences of adrenal medulla pheochromocytomas and hyperplasia in the low-dose group. The sensitivity of the study in male mice to detect a carcinogenic effect was reduced by the low survival of the high-dose group associated with fighting. There was *no evidence of carcinogenic activity* of γ-butyrolactone in female $B6C3F_1$ mice given 262 or 525 mg/kg in corn oil.

A decreased incidence of hepatocellular neoplasms in dosed male mice and decreased incidences of mammary gland fibroadenomas and cysts and pituitary cysts in female rats were associated with the administration of γ-butyrolactone.

Synonyms: Dihydro-2(3H)-furanone (8CI) (9CI), 1,2-butanolide, butyrolactone, 1,4-butanolide, 4-butyrolactone, 4-hydroxybutanoic acid lactone, γ-hydroxybutyric acid cyclic ester, γ-hydroxybutyric acid lactone, γ-lactone 4-hydroxy-butanoic acid, butyric acid lactone, butyryl lactone, 4-hydroxybutyric acid lactone, tetrahydro-2-furanone, 4-butanolide, 4-deoxytetronic acid, γ-hydroxybutyrolactone

Report Date: March 1992

TR-407 Toxicology and Carcinogenesis Studies of C.I. Pigment Red 3 (CAS No. 2425-85-6) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

C.I. Pigment Red 3, a yellowish red solid, is widely used for coloring paints, inks, plastics, and rubber, and in textile printing. It is used in a wide range of consumer items such as wallpaper, typewriter ribbons, carbon paper, and art materials. Toxicology and carcinogenicity studies were conducted by feeding groups of F344/N rats and B6C3F$_1$ mice of each sex diets containing C.I. Pigment Red 3 (97% pure) for 2 weeks, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells.

2-Week Studies: Groups of five rats and five mice of each sex were given feed containing 0, 6,000, 12,500, 25,000, 50,000, or 100,000 ppm C.I. Pigment Red 3 for 2 weeks. No chemical-related deaths occurred in rats or mice. Final mean body weights of exposed rats and male mice were lower than controls; female mice that received 6,000 and 50,000 ppm had significantly increased final mean body weights compared to that of the controls. The feed consumption of treated rats and mice was slightly greater than that of the controls, suggesting that C.I. Pigment Red 3 had no adverse effects on the feed palatability. Dose-related decreases in erythrocyte counts and hematocrit values and an increase in reticulocyte counts were observed in rats. Changes in these parameters were observed in mice, but there were no clear, dose-related trends.

13-Week Studies: Groups of ten rats and ten mice of each sex were given feed containing 0, 3,000, 6,000, 12,500, 25,000, or 50,000 ppm C.I. Pigment Red 3 for 13 weeks. No chemical-related deaths were observed in rats or mice. The final mean body weights of exposed female rats were significantly lower than that of the controls; the final mean body weights of exposed male rats and exposed mice were similar to controls. There were significant increases in relative liver and kidney weights of exposed male rats. Increases in the relative liver weights in mice did not occur with a dose-related trend and thus they were not considered related to chemical administration. Sites for the toxicity of C.I. Pigment Red 3 were the bone marrow, kidney, liver, and spleen in rats. Lesions observed in rats included bone marrow hyperplasia, congestion and hematopoietic cell proliferation of the spleen, and iron-positive pigmentation of the spleen, kidney, and liver. Sites for the toxicity of C.I. Pigment Red 3 in mice were the liver, kidney, and spleen in males and the liver and spleen in females. Lesions noted among mice in the spleen were hematopoietic cell proliferation and iron-positive pigmentation. In the liver, there was hematopoietic cell proliferation in male and female mice. Cytomegaly occurred in the renal tubule epithelium of the male mouse kidney.

2-Year Studies: Doses selected for the 2-year feed studies were 0, 6,000, 12,500, and 25,000 ppm for rats and 0, 12,500, 25,000, and 50,000 ppm for mice. The dose selection for rats was based on body weight changes observed for females that received 50,000 ppm; the dose selection for mice was based on the lack of body weight depression or death at the doses tested during the 13-week studies. Concentrations higher than 50,000 ppm in the feed were not used because higher levels might have adversely affected the nutritional value of the diet during the 2-year studies.

Body Weight, Feed Consumption, Clinical Findings, and Survival in the 2-Year Studies: Final mean body weights for male rats that received 25,000 ppm, female rats that received 12,500 and 25,000 ppm, and male and female mice that received 50,000 ppm were more than 10% lower than those of the controls. Feed consumption of exposed rats and mice was similar to that of the controls. No clinical findings indicative of toxicity were observed in rats or mice. The survival of low-dose male rats was greater than that of the controls (0 ppm, 28/50; 6,000 ppm, 40/50; 12,500 ppm, 28/50; 25,000 ppm, 20/50). Survival of exposed female rats and exposed male mice was similar to the controls; the survival of high-dose female mice was significantly decreased compared to that of the controls (39/50, 37/50, 31/50, 25/50). The reduced survival in this dose group may have been due to the increased incidence of ovarian abscesses.

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: Benign adrenal pheochromocytomas were significantly increased in the 12,500 and 25,000 ppm groups of male rats compared to the controls (22/50, 29/50, 35/50, 34/50). However, malignant neoplasms were not increased in incidence (6/50, 7/50, 10/50, 4/50). The incidence of adrenal pheochromocytomas in dosed groups exceeded the range for NTP historical controls for feed studies (22%-48%), and the increased incidence of this neoplasm was attributed to C.I. Pigment Red 3 administration.

Squamous cell papillomas of the skin occurred with a positive trend in male rats (0/50, 4/50, 2/50, 6/50), and the incidence in the high-dose group was significantly greater than that of the controls. A poorly differentiated squamous cell carcinoma (diagnosed as carcinoma) was observed in a control male. The historical control rate for squamous cell papillomas in NTP feed studies is low (16/800 or 2%, range 0%-4%), and the higher incidence of this tumor in male rats may have been caused by the administration of C.I. Pigment Red 3.

Hepatocellular adenomas occurred with a positive trend in female rats, with a significantly greater incidence in the high-dose group than in the control group (0/50, 0/50, 1/50, 10/50). This neoplasm has occurred in only one historical control group in NTP feed studies (3/800, range 0%-6%), and the increase in hepatocellular adenomas in female rats was attributed to chemical administration.

Chemical-related nonneoplastic lesions observed in the livers of male and female rats included eosinophilic or mixed type foci of cellular alteration. Foci were often accompanied by angiectasis and cystic degeneration in males and by granulomas and cholesterol pigmentation

in females. Chronic nephropathy occurred with increased severity in exposed male and female rats. The lesions were more severe in males than in females. Other lesions considered secondary to renal disease included parathyroid gland hyperplasia, fibrous osteodystrophy of the bone, and mineralization of various organs (stomach, intestine, heart, and blood vessels). The increased incidence of hyperplasia of the transitional epithelium of the renal papilla observed in treated rats was considered to be part of the chronic nephropathy.

Zymbal's gland carcinoma incidences were marginally increased in the mid- and high-dose male rats (0/50, 0/50, 2/50, 3/50). The incidence in the high-dose group was outside the NTP historical control range (0%-4%), and the Zymbal's gland carcinomas may have been related to C.I. Pigment Red 3 administration.

Mononuclear cell leukemias, mammary gland fibroadenomas, and preputial gland/clitoral gland adenomas occurred at lower incidences in exposed male and female rats. The decrease in mononuclear cell leukemia was attributed to the direct effect of C.I. Pigment Red 3 or its metabolites on the mechanism responsible for inducing leukemias in aging rats, while the decreased incidence of mammary gland fibroadenomas might be attributed to decreased body weights in female rats. The cause of the decreased incidences of preputial and clitoral gland tumors is unknown.

Tubule adenomas of the renal cortex occurred at a significantly higher incidence in high-dose male mice than in controls (0 ppm, 0/50; 12,500 ppm, 0/50; 25,000 ppm, 0/50; 50,000 ppm, 6/50). Because this tumor occurred only in exposed males and was outside the range for NTP historical controls in feed studies (0%-2%), renal cortical tubule adenomas in male mice were considered to be related to the administration of C.I. Pigment Red 3.

Follicular cell adenoma of the thyroid gland occurred with a positive trend in male mice (0/50, 0/49, 1/50, 5/50). The incidence in the high-dose group was significantly greater than that in the controls. This chemical-related effect is supported by the increased incidence of follicular cell hyperplasia. Because the incidence of this tumor exceeded the range of the historical controls from NTP feed studies (0%-4%), the increase of follicular cell adenoma was attributed to chemical administration. Female mice receiving C.I. Pigment Red 3 had a significant increase in follicular cell hyperplasia but showed no increase in tumor incidence at this site.

Focal renal tubule hyperplasia and cystic hyperplasia occurred in exposed male mice but not in the controls. Cytomegaly (karyomegaly) of the renal tubule epithelium was seen in all treated male mice. The severity of the accompanying chronic nephropathy was increased in both male and female mice.

Genetic Toxicology: C.I. Pigment Red 3 was mutagenic in *Salmonella typhimurium* strains TA100 and TA98 in the presence of exogenous metabolic activation (S9); no increases in gene mutation were observed in strains TA1535 and TA1537, with or without S9. C.I. Pigment Red 3 did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in either the presence or the absence of S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *some evidence of carcinogenic activity* of C.I. Pigment Red 3 in male F344/N rats as exhibited by increased incidences of benign pheochromocytomas of the adrenal gland. The marginal increase in the incidences of squamous cell papillomas of the skin and Zymbal's gland carcinomas may have been related to C.I. Pigment Red 3 administration. There was *some evidence of carcinogenic activity* of C.I. Pigment Red 3 in female F344/N rats as indicated by the increased incidence of hepatocellular adenomas. There was *some evidence of carcinogenic activity* of C.I. Pigment Red 3 in male $B6C3F_1$ mice as exhibited by the increased incidences of tubule adenomas of the renal cortex and follicular cell adenomas of the thyroid gland. There was *no evidence of carcinogenic activity* of C.I. Pigment Red 3 in female $B6C3F_1$ mice that received 12,500, 25,000, or 50,000 ppm.

The incidences of mononuclear cell leukemia and preputial gland tumors in male rats and mononuclear cell leukemia, mammary gland fibroadenoma, and clitoral gland tumors in female rats were lower in the exposed groups. The incidences of liver foci were markedly increased in exposed male and female rats. The severity of chronic nephropathy was increased in male rats and to a lesser extent in female rats given C.I. Pigment Red 3. An increase in the severity of nephropathy was observed in male and female mice; cytomegaly (karyomegaly) of renal tubule epithelium was observed in male mice. Thyroid follicular cell hyperplasia occurred with an increased incidence in male and female mice receiving C.I. Pigment Red 3.

Synonyms: 2-Naphthalenol, 1-((4-methyl-2-nitrophenyl)-azo)-; Calcotone Toluidine Red YP; Fast Red A; Pigment Scarlet R; Recolite Fast Red RBL; Sengale Light Red B

Report Date: March 1992

TR-408 Toxicology and Carcinogenesis Studies of Mercuric Chloride (CAS No. 7487-94-7) in F344 Rats and $B6C3F_1$ Mice (Gavage Studies

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-409 Toxicology and Carcinogenesis Studies of Quercetin (CAS No. 117-39-5) in F344 Rats (Feed Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-410 Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in B6C3F$_1$ Mice (Inhalation Studies)

Naphthalene, a white, crystalline powder, is used as a moth repellent and in the manufacture of phthalic and anthranilic acids, naphthylamines, and synthetic resins. The 2-year studies were conducted by exposing groups of male and female B6C3F$_1$ mice to naphthalene (>99% pure) vapor for 6 hours daily, 5 days per week, for 104 weeks. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary cells.

2-Year Studies: Groups of male and female mice were exposed to atmospheres containing 0 (75 mice per group), 10 (75 mice per group), or 30 ppm (150 mice per group) naphthalene. Mice from each group were included for 14-day hematology evaluations (male: 0 ppm, 5 animals; 10 ppm, 4; 30 ppm, 10; female: 0 ppm, 4; 10 ppm, 5; 30 ppm, 10). Mean body weights of exposed mice were slightly lower than those of controls throughout the studies. Survival of male control mice was significantly less than that of exposed mice; the lower survival was the result of wound trauma and secondary infections related to fighting among the group-housed mice (0 ppm, 26/70, 37%; 10 ppm, 52/69, 75%; 30 ppm, 118/133, 89%). Survival of exposed female mice was similar to that of controls (59/69, 86%; 57/65, 88%; 102/135, 76%).

Neoplastic and Nonneoplastic Effects in the 2-Year Studies: No increase in tumor incidence related to naphthalene administration was observed in male mice. In females, the incidence of pulmonary alveolar/bronchiolar adenomas was significantly greater in the high-dose group than in the controls (5/69, 7%; 2/65, 3%; 28/135, 21%). One other high-dose female had an alveolar/bronchiolar carcinoma. The combined incidence of alveolar/bronchiolar adenomas and carcinomas in the high-dose females was above those for control female B6C3F$_1$ mice from NTP feed, water, and inhalation studies (91/1,166, 7.8%, range 0%-16%). These lung tumors were attributed to naphthalene exposure.

Nonneoplastic lesions attributed to naphthalene exposure were observed in the nose and lungs of mice of both sexes. In the nose, naphthalene exposure was associated with an increase in the incidence and severity of chronic inflammation, metaplasia of the olfactory epithelium, and hyperplasia of respiratory epithelium. Chronic inflammation in the lung was associated with chemical exposure.

Genetic Toxicology: Naphthalene was negative for the induction of gene mutations in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with and without exogenous metabolic activation (S9). In cytogenetic tests with Chinese hamster ovary cells, naphthalene induced sister chromatid exchanges with and without S9 activation. Exposure to naphthalene induced a significant increase in chromosomal aberrations in Chinese hamster ovary cells in the presence of S9.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity* of naphthalene in male B6C3F$_1$ mice exposed to 10 or 30 ppm. There was *some evidence of carcinogenic activity* of naphthalene in female B6C3F$_1$ mice, based on increased incidences of pulmonary alveolar/bronchiolar adenomas.

In both male and female mice, naphthalene caused increased incidences and severity of chronic inflammation, metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium in the nose and chronic inflammation in the lungs.

Synonyms: Naphthalin, Naphthene, Tar Camphor

Report Date: April 1992

TR-411 Toxicology and Carcinogenesis Studies of C.I. Pigment Red 23 (CAS No. 6471-49-4) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this Compendium was prepared.

TR-412 Toxicology and Carcinogenesis Studies of 4,4'-Diamino-2,2'-Stilbenedisulfonic Acid Disodium Salt (CAS No. 7336-20-1) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this Compendium was prepared.

TR-413 Toxicology and Carcinogenesis Studies of Ethylene Glycol (CAS No. 107-21-1) in B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this Compendium was prepared.

TR-414 Toxicology and Carcinogenesis Studies of Pentachloroanisole (CAS No. 1825-21-4) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this Compendium was prepared.

TR-415 Toxicology and Carcinogenesis Studies of Polysorbate 80 (CAS No. 9005-65-6) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Polysorbate 80 is a nonionic surfactant used widely as an additive in foods, pharmaceutical preparations, and cosmetics as an emulsifier, dispersant, or stabilizer. Toxicity and carcinogenicity studies were conducted by administering polysorbate 80 (which met all compendial specifications) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*.

14-Day Studies: Groups of five rats and five mice of each sex received diets containing 0, 3,000, 6,000, 12,500, 25,000, or 50,000 ppm polysorbate 80. All animals survived to the end of the studies. The mean body weight change of male rats that received 50,000 ppm was significantly lower than that of the controls. The mean body weight changes in all other groups of dosed rats and in all groups of dosed mice were similar to those of the respective controls. No clinical findings or changes in absolute or relative organ weights in rats or mice were related to polysorbate 80 administration.

13-Week Studies: Groups of 10 rats and 10 mice of each sex received diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm polysorbate 80. All animals survived to the end of the studies. The final mean body weights of dosed rats and mice were similar to those of the controls. No clinical findings, changes in absolute or relative organ weights, or gross or microscopic lesions in rats or mice were related to polysorbate 80 administration.

2-Year Studies: Doses for the 2-year studies were selected based on the lack of observed compound-related effects at the dose levels used in the 13-week studies. Groups of 60 rats and 60 mice of each sex received diets containing 0, 25,000, or 50,000 ppm polysorbate 80 for up to 103 weeks.

15-Month Interim Evaluations: Interim evaluations were performed on 7 to 10 rats and mice from each dose group at 15 months. There were no significant changes in absolute or relative organ weights. Incidences of hyperplasia and inflammation of the forestomach were increased in female mice that received 50,000 ppm. No other chemical-related lesions occurred in rats or male mice evaluated at 15 months.

Body Weights, Clinical Findings, and Survival in the 2-Year Studies: The mean body weights in male and female rats and male mice administered polysorbate 80 were similar to those of the controls throughout the studies. The final mean body weight of female mice receiving 50,000 ppm was 11% lower than that of the controls. No clinical findings were associated with administration of polysorbate 80. The survival of dosed male rats was lower than that of the controls (0 ppm, 29/50; 25,000 ppm, 18/50; 50,000 ppm, 18/50); the survival of dosed female rats and male and female mice was similar to that of the respective controls (female rats: 23/50, 25/50, 25/50; male mice: 33/49, 34/50, 32/50; female mice: 30/50, 28/50, 26/50).

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: The incidence of adrenal medulla pheochromocytoma was marginally increased in high-dose male rats (21/50, 19/50, 29/50). The incidence of hyperplasia of the adrenal medulla was increased in low-dose male rats but not in high-dose male rats (11/50, 22/50, 12/50).

No chemical-related increases in the incidences of neoplasms occurred in male or female mice. The incidences of squamous hyperplasia and inflammation of the forestomach were significantly increased in high-dose male and female mice; forestomach ulcers were significantly increased in high-dose females.

Genetic Toxicology: Polysorbate 80 was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with or without exogenous metabolic activation (S9).

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* for polysorbate 80 in male F344/N rats based on an increased incidence of pheochromocytomas of the adrenal medulla. There was *no evidence of carcinogenic activity* for polysorbate 80 in female F344/N rats or in male or female B6C3F$_1$ mice given 25,000 or 50,000 ppm.

Administration of polysorbate 80 was associated with inflammation and squamous hyperplasia of the forestomach in male and female mice, and with ulcers of the forestomach in female mice.

Synonyms: Glycol; sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivatives; polyoxyethylene (20) sorbitan mono-oleate; sorethytan (20) mono-oleate; polyethylene oxide sorbitan mono-oleate

Trade names: Alkamuls PSMO-20; Armotan PMO-20; Capmul POE-O; Drewmulse POE-SMO; Emsorb 2722; Glycosperse O-20; Glycosperse O20 Veg; Glycosperse O20X; Hetsorb O20; Industrol O20S; Laxan ESO; Liposorb O-20; Lonzest SMO-20; Montanox 80; Nikkol TO-10;

Protasorb O-20; Sorbitan mono-oleate polyoxyethylene; Sorlate; Tween 80; Monitan; Olothorb; Sorbimacrogol Oleate 300; T-Maz 80

Report Date: January 1992